STUDIES ON THE PLASTICITY OF DORMANCY AND ON AGING IN SWITCHGRASS SEEDS

by

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(ABSTRACT)

The dormancy of switchgrass (*Panicum virgatum* L.) seeds may be broken by a variety of treatments, including after-ripening and stratification. This study was conducted to investigate and characterize more systematically factors affecting both after-ripening and stratification effectiveness, and the aging that can occur concomitantly with after-ripening. More than one year of after-ripening at ambient temperature and humidity was necessary for germination of newly harvested seeds to increase from as low as 5% to around 80%. After-ripening was not accelerated at temperatures above ambient for seeds stored in paper bags, which permitted the loss of seed moisture at the increased temperatures. Both after-ripening and aging accelerated with increases in temperature (5 to 60°C) and seed moisture content (50 to 130 g kg^{-1}), except that there was evidence of a moisture optimum for after-ripening that shifted downward as temperature increased. For many seedlots, storage at 60°C and 50 g kg^{-1} seed moisture content for about 1 mo broke most of the dormancy and resulted in acceptably low numbers of abnormal (aged) seedlings. Decreases in germinability caused by post-stratification drying of switchgrass seeds (described herein as "reversion", in which the reverted seeds could be made germinable again by further stratification) increased as the desiccation increased. Revertibility decreased as stratification or after-ripening time increased. Stratification and after-ripening worked additively to release switchgrass seeds from dormancy. Reversion (germination with stratification minus germination after stratification followed by drying) may reveal seedlot differences and changes over time and moisture content that can not be seen otherwise. Imbibed, dormant seeds placed at 21 or 30°C were induced into deeper dormancy, as indicated by length of stratification needed to break the dormancy. Dormancy deepened more as storage temperature and time increased for imbibed seeds. There are transitional temperature and seed moisture ranges where opposing processes (aging vs. after-ripening, stratification vs. dormancy deepening) appeared to overlap or surpass one another. Switchgrass seeds, either on a single seed level, or on the population level, responded continuously to changing temperature and moisture conditions. Less aging was observed for switchgrass seeds stored in N_2. After-ripening of switchgrass seemed not to be influenced by N_2 or air. In sum, switchgrass is revealed to be remarkably plastic in its ability to move toward both greater germinability and greater dormancy.

Dedication

Peace and good luck

-----*To my son and motherland*

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Introduction and Literature Review

JUSTIFICATION

Switchgrass is a tall-growing, perennial, C_4 species native to North America. It is one of the most promising multipurpose forage grasses. It can fill the forage production gap in June, July, and August, when the yield of cool-season forage is typically low (Vassey et al., 1985). Great potential has also been shown by switchgrass as an energy crop, where it could serve as a feedstock for production of renewable biofuels. Acreage planted with switchgrass could expand dramatically in the near future, especially for this latter use. Switchgrass has also gained some interest as a soil conservation crop and as wildlife cover.

Easily planted by no-till methods, switchgrass tolerates low soil moisture and minimal nitrogen fertilizer input. It is persistent and more productive than cool-season grasses on low pH (as low as 5.0) and low fertility soils in Virginia. Establishing a good stand is the first and greatest challenge in using switchgrass. Establishment can be very problematic, however, because of seed dormancy. Neoteric seedlots (typically less than 1 yr old) may have dormancy such that only 5% or less of the seeds will germinate when planted into a warm soil.

Dormancy can be released by a not-well-understood process called after-ripening, which occurs while seeds are stored under dry conditions. It is generally known that dry and cool conditions reduce aging but are not favorable for after-ripening during prolonged storage. Switchgrass seeds may need 2 yr or more to after-ripen sufficiently for successful sowing when stored under ambient conditions. Under the wrong conditions, that same 2 yr storage could result in serious aging of a seedlot. It is important for seed producers to know how to achieve and maintain the maximum germinability by controlling temperature and seed moisture content during seed storage.

Dormancy of switchgrass can also be removed by exposing seeds to a relative short period of wet, cool conditions (usually referred as stratification). It is of value to find the stratification time needed for full release of switchgrass seeds from dormancy. Stratified seeds need to be dried for mechanical planting, and the influence of drying on the germinability of stratified switchgrass seeds is therefore of interest.

In addition to storing switchgrass at dry-cool, dry-warm, and wet-cool conditions, warm-wet treatments will provide information necessary to understand the whole story of switchgrass seeds' response to temperature and moisture changes in their environment.

OBJECTIVES

As often happens when science is being done, the objectives of this work evolved as it progressed. The retrospective objectives of this research on switchgrass seeds were to test the following hypotheses: (1) storage temperature and seed moisture content will influence switchgrass seed after-ripening and aging, (2) some combination of temperature and seed moisture content (and/or anoxia) can break dormancy in a minimum period of time without

causing significant aging, (3) some environmental conditions can induce or deepen dormancy in switchgrass seeds, and (4) prolonged after-ripening and/or stratification can make switchgrass "irreversibly" germinable.

LITERATURE REVIEW

Angevine and Chabot (1979) suggested that dormant seeds have environmentally cued mechanisms that decrease the probability of encountering unacceptable growth conditions following germination. Temperature is arguably the most important environmental variable responsible for synchronizing germination with conditions suitable for seeding establishment. Roberts (1988) recognized three separate physiological processes in seeds affected by temperature. First, temperature together with moisture content determines the rate of deterioration or aging, in all seeds; secondly, temperature affects the rate of dormancy loss in dry seeds and the patterns of dormancy change in moist seeds; and thirdly, in nondormant, wet seeds temperature determines the rate of germination.

After-ripening may be defined as changes that occur in seeds during dry storage as a result of which dormancy is lost and germination is improved. The rate at which after-ripening occurs is a function of the storage environment as well as time. The rate of after-ripening is most notably temperature-dependent. Low temperatures greatly retard the after-ripening process. In a detailed study of dormancy in rice, Roberts (1962) demonstrated a negative linear relationship between the logarithm of the period of dry storage required for 50% germination (mean dormancy period) and temperature. This relationship is described by the equation: logd=Kd-Cdt where d=mean dormancy period, t=temperature, Kd=the intercept constant, and Cd= the slope constant. Subsequent experiments revealed considerable variation in the depth of dormancy between rice cultivars, as indicated by differences in the intercept constant Kd, but little change in the slope constant Cd. The relative effects of temperature on the rate of after-ripening are constant within a species. The Q_{10} for the relation is typically from 2.5 to 3.8 (Roberts, 1988). Thus, the longer and warmer the storage environment, the greater the loss of dormancy. For example, in one seedlot of *Oryza glaberrima* at 11.2% moisture content, half the seeds became germinable after 65 days at 30°C, while only 20 days were required at 40°C (Ellis et al., 1983); and so the Q_{10} was close to 3.

The moisture content of a seed in storage or in dry soil is established by the surrounding atmosphere and is in equilibrium with ambient relative humidity. There is evidence (limited to a few species) that seed moisture content can influence the rate of loss of dormancy during after-ripening, at least over certain moisture ranges (Quail and Carter, 1969; Tokumasu et al., 1975; Ellis et al., 1983). The effect of seed water content on the rate of after-ripening has probably been underestimated because of the dramatic effect of increasing moisture content on the rate of loss of viability in seeds (Roberts and Ellis, 1989). Complications set in when seeds are held at moisture contents higher than the norm for dry seeds, and substantial loss of viability may ensue. Rapid deterioration of seeds by microorganisms may occur from 18 to 30% moisture content, particularly in the presence of oxygen (Bewley and Black, 1982). The rate of after-ripening is least for seeds stored at very low moisture contents (<8%). It is greatest for cereals with 11 to 15% moisture content (Roberts, 1962, 1988; Ellis et al., 1983).

The activity of various other physiological processes in seeds has also been related to water status (Vertucci and Leopold, 1986; Leopold and Vertucci, 1989). Three critical regions of water binding have been identified in seeds which correspond to the three parts of the reverse sigmoidal water sorption isotherms characteristic of desiccation tolerant (orthodox) seeds. Based on thermodynamic principles, it has been calculated that water is highly bound at moisture contents below 10%, weakly bound between 10 and 20%, and bound with negligible energy at still higher moisture (Vertucci and Leopold, 1984). Using moisture isotherms constructed for red rice seeds at 2 and 22°C, Leopold et al. (1989) calculated water binding enthalpies according to the Clausius-Clapeyron equation: $\Delta H = (R+T_1*T_2)/(T_1-T_2)*\ln(aw_1/aw_2)$ where R=the gas constant; T_1 and T_2 = two temperatures; aw_1 and aw_2 = the relative humidities at the two temperatures for a given water content. It was speculated that after-ripening may involve some non-enzymatic oxidative reactions which are inhibited at lower moisture contents by a rising free energy and at higher moisture contents by oxidative metabolism.

After-ripening is accelerated by oxygen-enriched atmospheres and delayed in oxygen-depleted ones in rice and wild oats (Roberts, 1962; Simmonds and Simpson, 1971). In rice, exposure to 100% oxygen approximately doubled the rate of after-ripening over that occurring in seeds intermittently gassed with N_2. Aerobic respiration, or at least oxidation, apparently is essential to the after-ripening process in these species. The metabolism and growth associated with germination require an active synthesis of ATP. One hypothesis proposed is that dormancy is imposed by a block in energy metabolism and is broken by increased availability of phosphate acceptors such as glucose, presumably via the hydrolysis of stored forms (Ross, 1984).

Paradoxically many chemical inhibitors of respiration can also promote germination of wet dormant seeds. Those observations led to the proposal that the alternate cyanide-insensitive respiration pathway is involved in the termination of dormancy (Hendricks and Taylorson, 1972). Other inhibitors of respiration that do not act directly on the electron transport chain can likewise bring about germination in some species, and this led to a proposal that the pentose phosphate pathway was involved in some crucial step (Roberts, 1973). However, Jones and Hall (1981) could find no evidence linking ethylene-stimulated dormancy breakage of *Spergula arvensis* with pentose phosphate pathway activity. Upadhyaya et al. (1982), on the other hand, concluded that, although alternative respiration is necessary for the azide-stimulated germination of dormant fresh wild oat seed, it does not have a role in the germination of after-ripened seed nor in genetically nondormant varieties. Attempts have been made to examine these metabolic events *in vivo* and in some cases to draw together the two hypotheses (Taylorson and Hendricks, 1977), but so far the situation remains unsolved.

Switchgrass dormancy can also be removed by exposing seeds to relative short periods of wet, cool conditions (usually referred as stratification) (Zarnstorff et al., 1994). The effects of stratification on seed dormancy in many species have been well-covered in a number of reviews and general texts (Nikolaeva, 1977; Bewley and Black, 1985; Mayer and Poljakoff-Mayber, 1989). The required period of stratification is related to the seeds' mechanism of dormancy. The species of *Acer* have a range of chilling requirements from 50 to 120 d. Those species of *Acer* that require longer chilling times have an embryo dormancy, whereas those which are satisfied by shorter time have a coat-imposed dormancy (Bewley and Black, 1982). In Macedonian pine (*Pinus peuce* Grisebach), the highest germination observed was achieved with the longest

duration of stratification (Mason et al., 1995).

Seed dormancy is highly plastic and can also be induced and maintained under various environmental conditions. Moist seeds, both dormant and nondormant, can be induced into dormancy by stressful environments like low oxygen/or high carbon dioxide levels (Harper, 1957; Roberts, 1972). Germinability may be lost by exposing seeds to prolonged white light, especially at high radiant flux densities, or to far-red light (Bartley and Frankland, 1982; Ellis et al., 1986). Dormancy of some species is also readily induced in moist aerobic conditions. In many species, especially summer annuals, high temperatures induce dormancy, which is opposite to the stratifying effect with low temperatures (Baskin and Baskin, 1977, 1987; Bouwmeester and Karssen, 1993). The rate of dormancy induction in imbibed *Rumex crispus* seeds increases steadily between 1.5°C and 15°C. In contrast, the efficacy of dormancy release decreases over this temperature range (Totterdell and Roberts, 1979). Although short periods of chilling at these temperatures caused a loss of innate dormancy, induced dormancy was evident after as few as 7 d at 15°C (Totterdell and Roberts, 1979). High temperatures induce dormancy even sooner after the start of imbibition. A 3-d imbibition period at 20°C maximizes the loss of innate dormancy of *Rumex crispus* seeds as measured by the germination after a 2-h temperature shift to 35°C. Longer imbibition periods reduce the response to the temperature shift, implying an induction of dormancy (Totterdell and Roberts, 1981). Extended incubation at low temperatures was also reported in other species with dormancy that can be released by chilling (Willemsen, 1975; Bouwmeester and Karssen, 1993). Different results of chilling can even be observed within a single seed population due to genetic differences (Probert et al., 1989; Probert, 1992). Although dormancy-breaking effects of chilling are common, especially among species adapted for spring germination, low temperature can induce dormancy, particularly in species such as winter annuals that germinate in autumn (Baskin and Baskin, 1986). In Japanese brome (*Bromus japonicus* Thunb.), secondary dormancy was induced by imbibition at 0°C (Haferkamp et al., 1994). Induced dormancy may be relieved by after-ripening and chemicals, as with innate dormancy (Symons et al., 1986).

The first observation that a plant hormone could significantly break seed dormancy was made with a gibberellin, GA_3 (Green and Helgeson, 1957). Further observation with applied GA_3 showed that it overcomes several blocks to germination within seeds and caryopses. At maturity, dormant embryos of *Avena fatua* cannot synthesize a GA-like substance. Their ability to synthesize such a substance is fully developed after 24 mo of after-ripening. This development of activity can be inhibited by (2-chloroethyl) trimethylammonium chloride (CCC), an inhibitor of GA synthesis (Simpson, 1966). In cases of prolonged dormancy, the action of GA in overcoming dormancy of wet seeds is always slow, and some cases may take weeks. This suggests that other limiting processes are involved, especially during the early stages of after-ripening (Karssen et al., 1989). It is unclear whether the natural role of GA is to overcome dormancy, or, after dormancy is terminated by some other agent, to mobilize endosperm reserves. Although it is a well-reported hypothesis, there is no convincing evidence yet that a balance of GAs and inhibitors controls dormancy (Trewavas, 1981). An important action of GA in breaking dormancy may be modification of osmotic potential, achieved through promotion of formation of low molecular weight mono- and di-saccharides within the embryo and in the surrounding endosperm. It has been demonstrated in *Themeda triandra* that the hulls create a mechanical restraint on the

emergence of the radicle (Martin, 1975). A similar constraint can be achieved by decreasing the water potential of the germination medium. Both of these constraints can be overcome by the application of GA.

The most significant chemical inhibitors of germination that have been identified with certainty in various species belong to the family of abscisins. *Cis*, *trans* abscisic acid (ABA) has been identified in both primary and secondary florets of *Avena fatua* (Quail, 1968; Berrie et al., 1979), *Avena sterilis* (Tal, 1977), and the hulls of *Avena sativa* (Ruediger, 1982). However, the amounts found did not have an obvious relationship to the presence of dormancy. ABA has a powerful inhibitory effect on the germination of nondormant embryos (Andrews and Burrows, 1972; Cuming and Osborne, 1978) and intact florets (Holm and Miller, 1972). ABA can reverse the GA_3-simulated germination of dormant *Avena ludoviciana* (Quail, 1968). Both free and conjugated forms of ABA decline during after-ripening in *Zizania aquatica* as dormancy is lost (Albrecht et al., 1979). The embryo and pericarp contain 50 times the amounts of ABA found in the hulls and endosperm, and amounts are much less in nondormant than dormant seeds. A natural inhibitor, identified as ABA (Hayashi, 1979), decreases with after-ripening in both the hull and caryopses of dormant *Oryza sativa* (Hayashi and Himeno, 1973). Walker-Simmons et al. (1989) studied the specific biochemical process of germination that respond to ABA in wheat. ABA-treated embryos from mature dormant grain continuously synthesize acid-soluble protein for over 48 hr post-imbibition. Control embryos incubated in water do not. Two-dimensional protein analysis indicate that synthesis of a large number of acid-soluble proteins is regulated by ABA in embryos from dormant grains.

Despite the numerous papers published, the precise mechanisms of dormancy breaking and induction are largely unknown. Certainly, no universal or ubiquitous mechanisms (or even ones that apply to a truly wide range of species) have been described. Simpson (1978) pointed out that most of the experimental approaches to understanding the nature of dormancy have been based on the reductionist approach. Using this approach, analysis down to the level of constituent molecules is expected to "explain" seed dormancy. It is worthwhile, however, to realize that a useful description of dormancy may also be found in the holistic general view of the seed system (Simpson, 1990).

In the work that follows, a case is built for the remarkable plasticity and reversibility of dormancy-germinability in switchgrass. The evidence reported will suggest that switchgrass seeds at any point in time will exist somewhere along a continuum of dormancy-germinability and that they can be readily moved toward a more dormant or more germinable state. Seeds that are demonstrably germinable can be made dormant, and seeds that are marginally dormant can be made more deeply dormant. These shifts along the dormancy-germinability continuum have interesting practical and biological implications.

Seed aging will also be considered, which is presumably a unidirectional move across a continuum from high vigor/viability to low vigor/death. Because aging and dormancy-germinability shifts not only can but almost certainly will occur simultaneously, it is difficult to isolate their effects. Attempts will be made in treatments and discussions to separate the two.

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Chapter Two

Switchgrass Seed Dormancy and Aging as Influenced by Temperature during Storage in Paper Bags

ABSTRACT

Newly harvested switchgrass (*Panicum virgatum* L.) seeds are often highly dormant (less than 5% germinable when planted into a warm seedbed). Plantings of switchgrass, may fail due to the dormancy of seeds that are harvested in early fall and stored in unheated facilities during winter and early spring, which is the general practice but one that prolongs dormancy. Storage under other conditions might hasten after-ripening (dormancy-breaking) processes. More than one year of after-ripening at room temperatures is typically necessary for seed germination to reach 80 to 90%. The purpose of this research was to see if the dormancy of switchgrass seeds could be broken faster when stored under elevated temperatures. Seeds of "Cave-in-Rock" switchgrass harvested in 1992, 1993, and 1994 from the Midwest and Virginia were stored in paper bags at 5, 21, 30, and 45°C. Basal germination, germination following stratification at 5 °C for 2 wk, and germination following a post-stratification drying were observed for seeds sampled at various times during storage. After-ripening was not significantly accelerated at temperatures above ambient for seeds stored in paper bags. The number of abnormal seedlings increased with storage time and temperature. After more than 1.5 yr, seeds kept in paper bags at 45°C had a sharp decrease in germinability because of aging. Differences in germinability were observed among seedlots. At different storage times and temperatures, seedlots that after-ripened earlier also tended to age earlier. Post-stratification drying of seeds caused germination upon rewetting to decrease by half in some cases; however, this reduction is reversible with further stratification. Observing germination after stratification and germination after stratification followed by drying may reveal seedlot differences and changes over time not seen otherwise. It is recommended to purchase new seedlots of switchgrass seeds early and store them at room temperature or slightly above to enhance after-ripening. Elevated temperatures (above room temperature) may not hasten after-ripening of seeds stored in bins or porous bags, apparently because the higher temperatures lower seed moisture content and retard after-ripening.

INTRODUCTION

Dormancy of switchgrass seeds can cause establishment failures of this very promising multipurpose species. Neoteric (freshly harvested) seedlots may be highly viable but have less than 5% of their seeds germinable under temperature and moisture conditions that will allow nondormant switchgrass seeds to germinate. Such dormancy can be removed by after-ripening, which is the not-well-understood phenomenon of dormancy release that occurs during dry storage. After-ripening of some seeds has a relatively high Q_{10} of 2 to 4 (Roberts, 1965; Probert, 1992), offering the possibility of storage at moderate temperatures to hasten dormancy break. However, switchgrass seeds harvested in the fall are usually stored in outdoor or unheated areas by seed producers and farmers. As a result, the seeds may not after-ripen significantly before

spring planting.

Most reports on the influence of temperature on after-ripening of dormant seeds focus on temperatures below or slightly higher than room temperatures (Jensen, 1985). Several months to one year of storage at room temperature can result in much higher germination compared with switchgrass seeds stored at 0°C (Byers, 1973; Zarnstorff et al., 1994). Germination of "Cave-in-Rock" switchgrass increased from 35% to 85% after 23 wk of storage at room temperature (Aho et al., 1989). It could be a relatively simple practice for seedsmen and farmers to place seeds in a moderately heated storage environment to hasten dormancy break.

The aging phenomenon might bring complications to such an after-ripening treatment, however, because aging can also be enhanced by elevated storage temperatures (Ibrahim and Roberts, 1983). Germination of switchgrass seeds stored at 23°C generally started to decline after 180 d to 2 yr (Zarnstorff et al., 1994). Almost all research on aging deals with seeds that do not exhibit dormancy (Ellis and Roberts, 1981; Priestley, 1986). Reports are lack on the performance of dormant seeds that examine the opposing, concurrent influences of after-ripening and aging that might occur at higher-than-room temperatures.

Dormancy of switchgrass can also be removed by exposing seeds to a relative short period of wet, cool conditions (usually referred to as stratification) (Zarnstorff et al., 1994). The effects of stratification on seed dormancy in many species have been well-covered in a number of reviews and general texts (Nikolaeva, 1977; Bewley and Black, 1982; Mayer and Poljakoff-Mayber, 1989). The required period of stratification often varies with species and is related to the nature or depth of the seeds' dormancy. Various species of *Acer* have a range of chilling requirements from 50 to 120 days. Those species of *Acer* that require longer exposure times to chilling have an embryo-imposed dormancy, whereas those which are satisfied by shorter times have a coat-imposed dormancy (Bewley and Black, 1982). In Macedonian pine (*Pinus peuce* Grisebach), the highest germination was achieved with the longest duration of stratification (Mason et al., 1995).

Stratification can be used to break the dormancy of switchgrass seeds, however, the seeds need to be dried for mechanical planting. Emmerich and Hardegree (1996) reported that dehydration of imbibed seeds significantly reduced the subsequent germination of four warm-season grasses. The influence of drying on the germinability of stratified switchgrass seeds has not been reported. In preliminary studies, it was observed that the germinability of switchgrass seeds declines when they are dried after stratification. This decrease of germinability is not a result of seed death, because most of those seeds will become germinable when re-stratified. The reappearance of dormancy during post-stratification drying does complicate the use of stratification as a pre-planting treatment, however.

It is firmly established that seeds held in open storage deteriorate more slowly in cooler and drier localities than in warmer, more humid ones (Duvel, 1904). At increased temperatures, air can develop increased water vapor pressures. However relative humidity, which describes the percent of water vapor saturation, can and typically does fall as temperature increases; and relative humidity, in essence, largely drives the potential of seeds to absorb or desorb water. The equilibrium moisture content of seeds also depends to some degree on seed temperature, where higher temperatures reduce seed moisture content at any constant relative humidity. As a result, seeds stored in containers that are permeable to water vapor, will typically come to lower equilibrium seed moisture contents in warmer environments than seeds held at cooler

temperatures or seeds held in nonporous containers. It would be best to control both temperature and seed moisture content in seed storage. For seedsmen and farmers, however, it is generally easier to maintain temperature than moisture.

At least over some moisture range, there is a direct relation between seed moisture and the rates of after-ripening and aging (Probert, 1992; Riis et al., 1989). Seed aging studies in open storage are not as numerous as those where moisture is controlled or fixed. Robocker et al. (1953) found that germination of switchgrass stored at 23°C in open-air storage increased from 43 to 69% in the first year as seeds after-ripened but declined to 56% after a second year of storage, apparently as seeds aged and died. More needs to be known about dormancy changes in open environments with different temperatures, especially as those temperatures may alter seed moisture content, which can impact after-ripening and aging.

This research is designed to quantify changes in switchgrass seed dormancy and germinability that occur as a result of after-ripening and aging when seeds are stored in paper bags at a range of temperatures.

MATERIALS AND METHODS

Seedlot Sources

Seeds (more precisely, caryopses enclosed in an extrafloral palea and lemma) of "Cave-in-Rock" switchgrass were used throughout these studies. They typically exhibit a high degree of dormancy (usually less than 5% germination) when newly harvested. Seedlots harvested in 1993 included 10-93 from a Midwest commercial source (Sharpe Brother's Seed Company, Healy, KS 67850), 11-93 and 12-93 from another Midwest source (Osenbaugh Grass Seeds, Lucas, IO 50151), 1-93 from Blacksburg, VA, and 2-93 from Orange, VA. Seedlots harvested in 1994 included 13-94 purchased from Osenbaugh Grass Seeds and 1-94 from Orange, VA. Seedlot 1-92 was composed of various seedlots of 1992-harvested seeds that were naturally after-ripened during storage at room temperature for 2 yr. Seedlots from the commercial sources were held at 5°C after purchase. Seedlots from VA were also stored at 5°C after being hand-harvested in September. An air separator was used to clean all seedlots, so that average seed weight was greater than 1.85 mg/seed (540,000 seeds/kg).

Storage

Two series of storage trials were conducted. The 1993 series began in mid-November 1993 with five neoteric seedlots: 1-93, 2-93, 10-93, 11-93, and 12-93. The 1994 series actually began in mid-January 1995 with seedlots 1-92, 1-93, 12-93, 1-94, and 13-94. In each series, subsamples of approximately 40 g of each seedlot were placed in paper bags (coin envelopes) for storage at four temperature levels. A refrigeration unit with air circulation was used to maintain 5±1°C. In the 1993 study, one set of subsamples was kept at room temperature on a laboratory bench. In the 1994 study, a small incubator was used to create a constant temperature (21±1°C) close to room temperature. In both trials, an incubator was used for 30°C storage, and a "Snackmaster" dehydrator (Alternative Pioneering Systems, Inc., Chaska, MN 55378), which has

air circulation, was used for 45°C storage after adding more insulation to stabilize its temperature. Seed samples were taken for various germination tests after storage for 0, 3, 5, 8, 13, 14, 20, or 27 mo in the 1993 series, and after storage for 0, 2, 4, 6, 13, or 17 mo in 1994 series.

Seed moisture content (MC) was determined at various times for subsamples stored at each temperature. Seeds were weighed, dried in a 95°C oven for 36 hr, and reweighed. Seed moisture content= (Fresh Wt-Dry Wt)/Dry Wt.

Post-Storage Treatments and Germination Tests

Upon removal from storage after various times, subsamples of 100 seeds were placed in rolled, wetted germination toweling ("ragdolls") and tested for their germinability. The towels weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in 17.7 by 20.3 cm plastic bags with a zip closure. Fifteen to 18 ragdolls were placed in one plastic bag for subsequent treatments (either stratification or germination). No illumination was used during stratification or germination. (Preliminary studies showed that the germination percentage of switchgrass was not influenced by light).

Different treatment sequences were used before observing germination responses. Basal germination (G) was the value obtained by moving ragdolls immediately to 30°C for 10 d. Germination following stratification (SG) was determined by placing ragdolls at 5°C for 14 d or more and then transferring them to 30°C for 10 d. Germination following stratification and drying (SDG) involved stratifying seeds in ragdolls at 5°C for 14 d or more, drying in the ragdolls at room-temperature in moving air for 3 d, and then germination at 30°C for 10 d after rewetting. Seeds from the SDG treatment reached equilibrium with air and typically held less than 100 g kg^{-1} water after 3 d drying at room temperature in moving air. In the 1993 study, stratification periods up to 56 d were applied to observe SG and SDG before the start of storage. For seeds stored for varying times in both the 1993 and 1994 studis, the stratification was for 14 d at 5°C. In all cases (G, SG, and SDG), one count of germinants was done at 3 d to record and remove the early-germinating seeds, and a final count was made after 10 d at 30°C. Seedlings were considered normal germinants (NG) when both coleoptile and radicle extended at least 5 mm. If the coleoptiles or radicles protruded through the lemma and palea but failed to grow at least 5 mm, the seedlings were scored as abnormal germinants (AG). Total germination (TG) includes both normal and abnormal germinants (TG=NG+AG). When not otherwise indicated, germination values (G, SG, and SDG) reported include both normal and abnormal seedlings. Tetrazolium testing was not used to observe seed viability due to inconsistency of results. The reappearance of dormancy that we often observed following drying of stratified seeds is herein termed "reversion" (R) and is calculated as: R=SG-SDG.

Data Analysis

Analyses of variance for all germination variables were done with PROC GLM of SAS (SAS Institute, 1988). Germination data were converted to their square root and then arcsin transformed to comply with the basic assumptions of analysis of variance; back-transformed data

(sin transformation followed by square) are used for reporting. Results are reported as significant when the P-value was 0.05 or less for Tukey's Studentized Range (HSD) test. When analyzing the relationship between germination and storage time in the longer 1993 study, germination values of the seedlots from the same state, i.e., two seedlots from Virginia (1-93 and 2-93) and two seedlots from Iowa (11-93 and 12-93) were combined. Preliminary analysis showed that the combined seedlots were not significantly different. The resulting germination values from Virginia, Iowa, and Kansas (10-93) were considered as random variables and were analyzed by polynomial regression.

RESULTS AND DISCUSSION

1993 Study

Pre-storage characterization of seedlots: responses to duration of stratification and to post-stratification drying

No significant differences between seedlots were observed for basal germination (G) (without stratification) at the beginning of the study (Table 2.1); all five seedlots showed no more than 7% germination. Two wk of stratification brought germination (SG) up to 60 to 80%. Seedlot 2-93 had the highest germination with 2 wk of stratification, while 11-93 and 12-93 were lower. Mean germination for all seedlots with up to 8 wk of stratification remained around 65 to 80% and very similar to that following 4 wk. From 15 to 35% of the seeds (depending on seedlot) were either still dormant or dead with stratification as long as 8 wk. Seedlots 11-93 and 12-93 from Iowa showed germination percentages that were (or tended to be) lower than seedlots 1-93 and 2-93 from Virginia with all stratification durations. Seedlot 10-93 from Kansas was intermediate in germination. Only SG of 1-93 and 2-93 increased quadratically between 2 to 6 wk of stratification. SG for the three Midwest seedlots (10-93, 11-93, and 12-93) as well as for the mean did not change significantly during prolonged stratification. The Midwest seedlots probably had some dead seeds already. The differences between seedlots in germination responses to stratification may be caused by their growing environment or production history.

The highest germinations after 2 or 4 wk of stratification followed by drying (SDG) were seen with seedlot 2-93. After 6 wk of stratification, seedlot 12-93 had a lower SDG than 1-93 and 2-93. No difference was observed for SDG of seedlots after 8 wk of stratification. For each seedlot and the mean, SDG continued to increase through 8 wk of stratification. SDG therefore appeared to reveal something about the physiological state of the seeds that SG did not, since SG differences were not evident within seedlots after 4 wk of stratification.

Nearly half of the seeds that were made germinable by 2 wk of stratification would revert to a dormant state when dried. This reversion (R) decreased as stratification time extended from 2 to 8 wk. No difference was observed for R between seedlots except at 4 wk of stratification, when 11-93 showed less reversion than 1-93. Other experiments (see Chapter 4) will show that the germination decrease caused by drying is recoverable if seeds are stratified for another 2 wk. It is clear, then, that the reduction of germination during drying is not caused by death of seeds. While stratification for 2 wk can break the dormancy of most seeds, prolonged stratification was

needed to prevent a portion of those seeds from reverting to dormancy when dried.

It may be significant that some seedlots, especially 11-93 and 12-93 only attained a maximum germination of about 70% with any duration of stratification tested. These Iowa seedlots were either "deeply dormant", had a high percentage of seeds non-responsive to stratification, or had many dead seeds.

The influence of storage temperature and time on germination, stratification, and reversion

The fitted polynomial curves for germination of the five seedlots from the 1993 study varied with storage temperature (Fig. 2.1). The fitted curves, at least at the higher two storage temperatures, reflected a rise as dormancy was broken and then a fall presumably due to aging. In Fig. 2.1, area I (above SG) represents seeds which were either dead or dormant and not made germinable by stratification at 5°C for 2 wk. The area II (between SG and SDG) reveals those seeds that reverted into a dormant status if stratification was followed by drying, i.e., R. The area III (between SDG and G) denotes those seeds whose dormancy was broken by 2 wk of stratification and that remained germinable even after drying. The area IV (below G) shows seeds that were inherently nondormant or that after-ripened sufficiently to become germinable without further treatment such as stratification. The combined area between SG and G (II+III) indicates the portion of the seeds that are dormant but can be made immediately germinable by stratifying 2 wk.

At 5°C storage, the increase in G with time, i.e., after-ripening, was very slow. The increase in G seemed to accelerate after one year storage. These data show why newly harvested seeds stored under cool conditions during winter and early spring will not be ready for sowing unless some additional treatment is applied.

In contrast to G, SG started at a rather high 72% and increased only about 8% in the first 12 mo and then leveled off at about 80%. On the other hand, SDG began at about 35% and increased linearly by 17% in the first 12 mo of storage at 5°C and another 12% to reach 64% in the following 15 mo. Reversion (R) following post-stratification drying decreased from 37% of the seeds at the beginning to 16% after 27 mo storage at 5°C. It is concluded therefore that some quality of the seed, which we shall call "the degree of dormancy" and which is revealed at least partially by reversion, changed during 5°C storage. (Reversion will be looked at more closely in Chapter 4.) Shifts in reversion (SDG) indicate things are happening in the seed even if germination (G) is not going up and the stratification response (SG) seems rather static.

The time needed to release seeds from dormancy, i.e., to after-ripen them, was least at 45°C storage. The peak values for SG, SDG and G seen in Fig.2.1 appeared sequentially and diverged in time more at 45°C than at lower storage temperatures. The appearance of peak values presumably reflected the time when the majority of the seeds had been released from dormancy but before many seeds had died. The earlier appearance of peak values at 45°C suggests that seeds after-ripening more rapidly and that few seeds were dying in the first few months of storage. The presence of reversion again suggests dormancy breaking is not an irreversible event and that after-ripening can overcome the tendency for seeds to revert. This also raises the possibility of a "continuum of dormancy-germinability" which seeds can move along in either direction. More evidence will be addressed for this notion throughout this dissertation.

At 21, 30, or 45°C, it took about 8 mo for seedlots to reach 50% germination without stratification (G). This suggests that the Q_{10} for after-ripening was close to one between 21 and 45°C. The increased temperature did not double or treble the after-ripening speed for each 10°C rise as might be expected from the literature. One explanation for the low apparent Q_{10} could come from the seed moisture decreases seen at higher temperatures when stored in the paper bags (Table 2.2). Due to vagaries of circulation and perhaps due to other materials stored in the chamber, seed moisture content (MC) at 5°C varied from 80 to 170 g kg^{-1} depending on sampling time. Seeds placed on the laboratory bench also varied in MC from 100 to 160 g kg^{-1} at different times. Relatively narrower ranges of MC were shown by seeds stored at 30 and 45°C. It is obvious from Table 2.2 that MC has confounded this experiment, but the study is still quite valid for predicting seed responses to storage in porous containers or bins. Although less has been reported about the influence of seed moisture on after-ripening, it has been well established that drier seeds generally age slower (Ellis and Roberts, 1980, 1981). Switchgrass seeds needed to be after-ripened for 6 to 8 mo around room temperature in this study to ensure minimally acceptable germination (more than 50%) at time of seeding. Zarnstorff et al. (1994), however, reported that post-harvest storage of seeds at 23°C from January to April (90 d) should ensure adequate germinability at time of seeding. It is perhaps significant that the germination tests of Zarnstorff et al. were done with an alternating temperature of 30/15°C, and we suspect that stratification, which breaks dormancy, may happen when seeds were "germinated" at 15°C. Zarnstorff et al. also were working with cultivars other than Cave-in-Rock. Our studies (data not shown) suggest Cave-in-Rock may be more dormancy prone than many other cultivars.

The highest values observed for germination and fitted curves were shown at 30°C and about 17 to 24 mo of storage. The observed values for 21°C had not yet started to decrease by 27 mo (the end of the study) and thus might have ultimately exceeded those highest values of 30°C. That the highest G value for 30°C did not approach 100% could mean that there were some dead seeds at the beginning of the study. (See earlier discussion of low SG seen with seedlots 11-93 and 12-93). It may also be the case that some seeds started to die before they or others are released from dormancy. The second explanation is more feasible for 45°C storage. Compared with 30°C, the peak for G was lower and then dropped more sharply for storage of 45°C. Although it took very similar times for G to reach 50% at 21, 30, and 45°C, it took 22, 21, and 18 mo for calculated G to peak, respectively. This suggests the influence of temperature was perhaps more manifested in aging than in after-ripening or that the confounding MC was affecting the two processes differently.

At the beginning of storage, SG and SDG were higher than G because stratification broke dormancy, albeit reversibly in some cases. For seeds stored at 45°C, peak germination values appeared 10 and 6 mo earlier for SG and SDG than for G. These observations are consistent with the notion that after-ripening plus stratification had an additive effect on dormancy break and that after-ripening had a cumulative effect on removing revertibility. Storage for 20 mo at 45°C decreased SG and SDG to 50%, but G had just started to decrease. After storing at 45°C for 27 mo, the values of SG and SDG were lower than G, i.e., stratification of those seeds decreased their germinability. This was likely because both stratification and post-stratification drying killed the now fully nondormant, already aged and weakened seeds. Evidence for aging in the 1993 study will be more closely examined in the following section.

The influence of storage temperature and time on the ratio of normal versus abnormal seedling

Changes in the germinability of a seedlot over time can be separated into two phases: dormancy breaking (or after-ripening) that increases germination, and aging that ultimately decreases germination. A diminution of biological activity, which results in abnormal seedlings, typically precedes seed death (Ellis and Roberts, 1981). At 14 mo storage in the 1993 study, there were few abnormal germinants (AG), but AG was significantly higher for seeds stored at 45°C (Fig. 2.2, above). At 20 mo, NG and TG for seeds stored at 5°C were significantly lower than at 21, 30, and 45°C. No differences were observed for AG between 21 and 30°C after 20 mo, but AG at 5°C was lower and at 45°C was higher. It can be concluded that aging, as indicated by AG, went up as storage temperature increased. More difference was seen in AG than in NG and TG. After 20 mo of storage, the influence of temperature on the aging process was more readily seen if AG was observed. The number of abnormal seedlings increased between 14 and 27 mo. With the decrease of NG for seeds stored at 21, 30, and 45°C, the ratio of abnormal seedlings increased.

No significant differences were shown for NG, AG, or TG after 14 mo of storage plus stratification for 2 wk (Fig. 2.2, below). After 20 mo plus stratification, AG for 45°C was significantly higher than AG for other temperatures. No difference was observed among 5, 21, or 30°C after 20 mo storage plus stratification. Both aging (reflected by AG) and death (reflected by a declining TG) caused by aging accelerated significantly at 45°C. The difference in aging was more evident with stratified seeds. Stratification itself might cause some stress to aged seeds. After 27 mo of storage at 45°C plus stratification, NG and TG were significantly different from those at other storage temperatures. Significantly more seeds died when stored for 27 mo at 45°C than at other temperatures. More than half of the seedlings were abnormal for seeds stored at 45°C for 27 mo and then stratified. Stratification was clearly harmful to aged, fully after-ripened seeds.

1994 Study

After-ripening (as measured by G) during storage at different temperatures

Only seedlot 1-92, which was extensively after-ripened during 2 yr storage at room temperature, had substantial germinability at the beginning at the 1994 study (Table 2.3). No differences were observed in G (ranging from 3 to 7%) for the four more nearly neoteric seedlots. This did not mean, however, that the four seedlots other than 1-92 were at the same status of dormancy. After 2 mo at 30°C, G of seedlot 12-93 had increased such that it was significantly higher than 1-93, 1-94, and 13-94. After 2 mo at 45°C, G of 12-93 was also higher than 1-94 and 13-94.

After 6 mo storage at 5°C, G had not increased notably (Table 2.3). At 21°C, the G for 13-94 was significantly lower than 1-94, which was itself lower than 1-93 and 12-93. The most recently harvested (1994) seeds apparently after-ripened more slowly than the 1993 seeds that had been stored for 1 yr at 5°C. Seedlots harvested in the same year may have had a different rate of

after-ripening also. At 45°C for 6 mo, the G of seedlot 1-93 exceeded that of 12-93 and 1-94 exceed 13-94. The G of 13-94 was still significantly lower than all the others when stored at 45°C for 6 mo. Except for seedlot 1-93, there appeared to be no greater after-ripening at 45°C than at 30°C.

For the four seedlots that were more or less neoteric, G at 5°C for 13 mo was somewhat higher than at 6 mo (Table 2.3). With 13 mo at 21°C, there was no longer a significant difference between 1-92 and either 1-93 or 1-94. The G of 12-93 and 13-94 were significantly lower than for the others. The lower G of 13-94 was attributed to insufficient or incomplete after-ripening, while 12-93 seemed atypical or aberrant. The lowered G of 12-93 and 1-92 at 45°C and 13 mo (relative to 30°C and 13 mo) was almost certainly due to aging-related death. It would appear that 1-92 and perhaps 12-93 were already somewhat aged as well as more after-ripened at the beginning of the study. Thus they may have succumbed more quickly to the less favorable high storage temperature.

Storage for 17 mo at 5°C differentiated seedlots in their G, and 12-93 was clearly after-ripening more quickly than the other 1993 or 1994 seedlots (Table 2.3). At 21°C, the G of 1-93 was very high (92%) and significantly higher than all the other seedlots. At 30°C, 12-93 was significantly lower in G than other seedlots but near the maximum it ever attained. Storage at 45°C for 17 mo lowered the G of 1-92 and 12-93; both had higher germinability at the earlier times, and they now decreased in G below those of other seedlots. The G of 12-93, however, was significantly lower than 1-92. This means the seedlot with the highest germination at the beginning was not necessarily the one that aged the most quickly. Seedlot 12-93 again gives evidence that it may have had many dead or imperfect "seeds" at the outset, since its G never got much above 70% even in the 1993 study. It also gave evidence of more rapid aging and/or sensitivity to higher storage temperatures.

The change of G over storage temperature at 6, 13, and 17 mo of storage could be described by quadratic equations for all seedlots. Seedlots 1-93, 1-94, and 13-94 increased in G as temperature went up from 6 to 13 mo of storage. After 17 mo of storage, all seedlots has a G decrease as temperature increased from 30 to 45°C (Table 2.3).

Multiple regressions showed that G was influenced significantly by storage time, temperature, and their interaction (Table 2.3). The G differences across temperatures were greater at later storage times for all seedlots except 12-93. With the storage time increased, $\beta 1$ and $\beta 2$ tended to increase also, indicating that the curvature of fitted regression curves, thus the response of G to temperature difference, increased. Smaller $\beta 1$ and $\beta 2$ factors for 1-92 also showed that the G of this highly germinable seedlot was less influenced by temperature compared with other seedlots. 1-92 was not fully after-ripened at the outset of the study, but it was perhaps nearly so.

The influence of after-ripening at different temperatures on SG of various seedlots

Multiple regressions showed that the SG of some seedlots was influenced significantly by storage time, temperature, and the interaction between time and temperature (Table 2.4). Seedlot 1-93 SG was surprisingly high and did not change significantly over time, while seedlot 1-94 did not change SG significantly over time or temperature. The change of SG over storage

temperature at 2, 6, or 13 mo was not significant for most seedlots (Table 2.4), i.e., the temperature of storage did not have a significant influence on SG at the early storage times. All these data suggest that any dormancy of the seedlots could be readily overcome by stratification for 2 wk even without after-ripening.

At 13 mo, seedlot 13-94 showed an increase in SG as storage temperature increased from 5 to 30°C but a decrease when storage temperature increased to 45°C. After 17 mo, all seedlots showed a decrease in SG when the storage temperature was 45°C. After 17 mo at 45°C, SG was lower than G, indicating stratification was an additional stress to seeds already aged and weakened by long storage at high temperatures. Temperature of storage influenced aging of seeds at longer storage times but not stratifiability at earlier storage times.

The highest G ever reached by 12-93 was 73%, which was lower than that of other seedlots. Was it a seedlot with low vigor and germination from the beginning of storage study? The SG for 12-93 at the beginning of storage was 73% (Table 2.4). Several combinations of temperature and storage time resulted in SG around 80% for 12-93, a lower maximum germinability than observed in the other seedlots and well below the mean maximum at 91%. So probably seedlot 12-93 had relatively lower potential germinability at the beginning. Other seedlots were similar to seedlots 12-93 in that the highest values for G were lower than those for SG and SDG (Table 2.4, 2.5, 2.6). Some seeds apparently aged and died before the last seeds were released from dormancy by after-ripening, or they may have been dead from the outset.

Seedlots 13-94 and 12-93 had the lowest SG at the beginning (Table 2.4). After 17 mo at 30°C, 13-94 reached its highest observed SG of 94%. Prolonged after-ripening seemed to make seeds more susceptible to dormancy break by 2 wk of stratification at 5°C. SG of 1-93 increased significantly during after-ripening. SG of 1-92 also increased and decreased significantly during 17 mo of after-ripening. Even for this highly germinable seedlot at the very beginning, there apparently were seeds that could not be released from dormancy by 2 wk of stratification at 5°C. Only after additional after-ripening could these seeds be rendered germinable by 2 wk of stratification. This provides more evidence for a "depth of dormancy" concept, in which seeds may be so deeply dormant or non-germinable that extended periods of after-ripening (and/or stratification) are needed to move them sufficiently across the continuum that they become germinable.

The influence of after-ripening at different temperatures on SDG of various seedlots

Multiple regressions showed that SDG of most seedlots was more sharply influenced by storage time and temperature than was SG (Tables 2.4 and 2.5). Time and temperature interacted for seedlots 1-92 and 12-93. Except for 1-92 at 6 mo, the SDG of all seedlots changed significantly across storage temperatures after 6, 13, or 17 mo of storage. At all storage times, the increase in SDG over storage temperatures was greater than SG but less than G. Also SDG increased more slowly than SG but more quickly than G (Table 2.3, Table 2.4, Table 2.5). All seedlots at 13 and 17 mo of storage and all but two seedlots at 6 mo of storage showed a significant decrease in SDG as storage temperature increased from 30 to 45°C. SDG showed an earlier decrease than G or even SG at the higher temperatures. The stress inherent in drying may cause SDG to decrease earlier than SG for seeds stored (and aged) at higher temperatures.

Seedlots 1-92 and 1-93 were significantly higher in SDG at the beginning of storage. Seedlot 12-93 was also higher in SDG than the two 1994 seedlots at the beginning of storage (Table 2.5). SDG of all seeds increased and then decreased as storage time increased. Although both seedlots 1-92 and 1-93 had quite high SDG at the beginning of experiment, i.e., they were well after-ripened, there still was a small portion of their seeds that remained in a dormancy state that could be broken by 2 wk of stratification but that would revert to dormancy when seeds were dried. Between 6 and 17 mo of storage, there were significant decreases of SDG in 1-92 and 12-93 at 45°C. During that time, the decreases in SDG were also seen with storage at 21 and 30°C. Seedlot 12-93 decreased most dramatically in SDG between 13 and 17 mo. At 17 mo and 45°C, the lowest SDG was 15% for 12-93 and the second lowest was 31% for 1-92. The aging influence was most evident at later times and high temperatures for seedlots with history of long storage and that were therefore probably more aged at the beginning of experiment (Table 2.5).

At the beginning of storage, there was no significant difference in SDG between 1-94 and 13-94. After 2 mo at 30 or 45°C, SDG of seedlot 1-94 was higher than for 13-94. This same difference appeared for 5°C storage after 6 mo of storage. With further storage, the differences disappeared again for the two seedlots (Table 2.5). The differences in G between 1-94 and 13-94 followed the same pattern but several months later (Table 2.3). The difference in SG between 1-94 and 13-94 appeared at the beginning and remained only for 30°C after 2 mo (Table 2.4). This suggested that changes in G followed SDG, which followed with SG. Seedlot 1-93 was persistently higher than 12-93 in SDG and SG (Table 2.5). This may be attributed to the aging and death of 12-93 seeds or to an inherently lower number of viable seeds. The difference in G, however, followed the same pattern as between 1-94 and 13-94.

Changes in R during storage at different temperatures

Subtracting SDG (Table 2.5) from SG (Table 2.4) reveals the number of seeds in each seedlot that lose germinability during post-stratification drying (Table 2.6). Although no difference was seen in G between 1993 seedlots and the newly harvested 1994 seedlots, they indeed were in a different status of dormancy as indicated by significant differences in R (Table 2.6). Seedlots 1-94 and 13-94 showed significantly more reversion than the other seedlots at the beginning of storage. With storage of 4 to 13 mo, there was no difference for R between seedlots at all temperatures except 5°C (Table 2.6). Seedlot 13-94 had more reversion than 1-92 and 1-93 at 6 mo at 5°C. Seedlots 1-92 and 12-93 also showed lower R than 13-94 at 13 mo and 5°C. Seedlots with the lowest SG seemed to have more seeds that would revert to dormancy if stratification was followed by drying. After 17 mo, there was no significant difference in R between seedlots (Table 2.6). The difference in R among seedlots disappeared earlier than in G (Table 2.3, Table 2.6).

R appeared to decrease quadratically over storage temperature at 6 and 17 mo storage but only linearly at 13 mo (Table 2.4). Storage at 5°C had the least effect on dormancy reversion, while the other three temperatures seemed to have larger effects. The change of R over time followed quadratic patterns for all seedlots except 1-92.The change of R over temperature was not significant for seedlots 1-92 and 1-94. The other three seedlots decreased linearly in R over storage temperature.

SUMMARY AND CONCLUSIONS

These data suggest clearly that the temperature to which seeds are exposed during storage influences their after-ripening rate. At 5°C, dormancy (without post-storage stratification) remained high. Seeds of a neoteric seedlot held at room temperature needed more than 1 yr to minimize dormancy reversion, which seems to be an indicator of some "semi-germinable" state along a putative dormancy-germinability continuum. More than 1 yr of after-ripening at 21°C was necessary for the seeds to break 80 to 90% of their dormancy. Similar times (9 mo) were needed for seedlots to reach 50% germination when they were stored in paper bags between 21 and 45°C. Temperatures higher than room temperature apparently failed to hasten after-ripening ($Q_{10} \approx 1$) due to seed moisture decreases. Seed producers might store switchgrass seeds in a heated warehouse and take efforts to maintain proper seed moisture content. Farmers who obtain neoteric switchgrass seeds in December or January will have difficulty breaking enough dormancy simply by storing seeds at room temperature before planting in April or May.

The number of abnormal seedlings increased with storage time and temperature. After more than 1.5 yr, seeds stored at 45°C showed a significant decrease in total germination because of aging. As early as 14 mo, normal seedlings developed by seeds stored at 45°C were fewer than at 30°C storage due to more aging at the higher temperature. Storage at room temperature for more than 2 yr could also cause aging that increases the number of abnormal germinants. Germination has increased but the ability of those no-longer dormant seeds to produce a vigorous seedling is declining due to aging.

Newly harvested "Cave-in-Rock" switchgrass seeds had germination less than 7%. Stratification at 5°C for 2 wk brought germination up to 60 to 80%. The germination of such stratified seeds often goes down to 30 to 50% when dried due to a previously undescribed phenomenon that we call reversion. That phenomenon will be examined more closely in subsequent chapters. While G and SG for newly harvested 1994 seedlots and for 1993 seedlots stored at 5°C for 1 yr were the same, the seedlots differed significantly in germination with stratification followed by drying (SDG). Dormancy reversion may reveal changes or a physiological status not seen otherwise.

Differences in dormancy status were also demonstrated between 1993 and 1994 seedlots at various times and temperatures for G, SG, and SDG. After-ripening varied with seedlot and storage temperature. Seedlots that increase in germination (after-ripen) earlier may also decrease (age) earlier. At different storage times and temperatures, different seedlots after-ripened and aged in an orderly or predictable way. Germination following storage for a specific time and at a specific temperature, especially without considering stratification and drying response, is not sufficient for comparing seedlots.

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Table 2.1. The influence of stratification time on germination of five neoteric switchgrass seedlots prior to extended storage (1993 study)

Stratification time	Germination after 10 d at 30°C, by seedlot					
	1-93	2-93	10-93	11-93	12-93	Mean
wk	----------%----------					
	Not stratified (G)					
0	3a†	5a	7a	3a	4a	4
	Stratified but not dried (SG)					
2	70abc	80a	77ab	66bc	62c	72
4	86ab	90a	79bc	72c	68c	80
6	89a	87a	77ab	63b	66b	77
8	86a	83ab	77ab	69ab	65b	76
β_1‡	14.2**	9.1*	-	-	-	-
β_2	-1.1*	0.90*	-	-	-	-
r^2	0.66**	0.48*	-	-	-	-
	Stratified and dried (SDG)					
2	38ab	49a	33b	28b	30b	35
4	49b	66a	48b	50b	45b	51
6	70a	74a	62ab	61ab	56b	65
8	80a	80a	68a	70a	64a	73
β_1	7.3**	12.0**	6.0**	15.0**	5.8**	6.2**
β_2	-	-0.65**	-	-0.79*	-	-
r^2	0.91**	0.96**	0.78**	0.95**	0.93**	0.73**
	Reversion of germinability (R) caused by drying (R=SG-SDG)					
2	32a	31a	44a	38a	32a	35
4	37a	25ab	31ab	22b	24ab	27
6	18a	12a	12a	12a	15a	14
8	12a	11a	15a	1a	3a	8
β_1	-4.9**	5.0**	-6.1**	-6.9**	-5.5**	-5.6**
r^2	0.60**	0.86**	0.72**	0.85**	0.81**	0.71**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Values within the same row followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

‡ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seedlot, where y is the germination of switchgrass seeds that had been stratified at 5°C for 2 wk without drying (SG), with drying (SDG), or germination decrease caused by drying following stratification (R), and x is the weeks of continuous stratification at 5°C. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted.

Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the equation.

Table 2.2. Water content of switchgrass seedlots stored at four temperatures.

Storage temperature	1993 Study		1994 Study	
	Water content†	Standard error	Water content	Standard error
°C	g kg^{-1}		g kg^{-1}	
5	165	3.5	131	1.3
21‡	100	1.8	158	0.9
30	77	1.3	75	1.4
45	39	1.9	41	1.6

†Means of five seedlots in each study.
‡Seedlots for the 1993 study were stored on a laboratory bench. In the 1994 study, a constant storage temperature (21±1°C) was used.

Table 2.3. Germination response of five switchgrass seedlots during after-ripening as influenced by time and temperature when stored in paper bags (1994 study).

Storage time	Storage temperature	Germination (G), by seedlot					
		1-92	1-93	12-93	1-94	13-94	Mean†
Months	°C	----------%----------					
0	-	77a‡	7b	4b	3b	3b	4
2	30	79a	10c	28b	9c	7c	13
	45	80a	13bc	27b	8c	4c	12
4	21	81a	28b	38b	26bc	12c	25
6	5	81a	6b	7b	5b	6b	6
	21	87a	58bc	64b	47c	30d	50
	30	88a	63b	62b	58bc	42c	57
	45	80a	76a	60b	58b	38c	58
β_1		0.90**	4.30**	5.20**	4.10**	2.60**	4.10**
β_2		-0.018**	-0.053**	-0.079**	-0.056**	-0.036**	-0.056**
r^2		0.61**	0.95**	0.91**	0.99**	0.92**	0.78**
13	5	79a	12bc	18b	8c	8c	11
	21	83a	84a	56b	75a	58b	69
	30	87a	88a	73a	82ab	80ab	81
	45	71c	93a	56d	80b	84b	80
β_1		1.10**	6.40**	4.60**	6.30**	5.00**	5.60**
β_2		-0.027**	-0.089**	-0.073**	-0.092**	-0.062**	-0.079**
r^2		0.64**	0.96**	0.95**	0.98**	0.99**	0.89**
17	5	81a	17c	32b	19c	9d	19
	21	83b	92a	54c	81b	78b	78
	30	86a	92a	69b	83a	87a	83
	45	64b	85a	44c	81a	82a	75
β_1		1.10*	7.00**	3.40**	5.70**	6.60**	5.70**
β_2		-0.031**	-0.110**	-0.060**	-0.084**	-0.097**	-0.087**
r^2		0.75**	0.96**	0.83**	0.96**	0.98**	0.79**
γ_1¶		1.00**	3.60**	3.30**	3.30**	2.50**	3.10**
γ_2		-0.019**	-0.062**	-0.053**	-0.058**	-0.049**	-0.056**
γ_3		1.80**	6.60**	8.40**	5.40**	2.40**	5.60**
γ_4		-0.074**	-0.290**	-0.350**	-0.022*	-0.094*	-0.240**
γ_5		-0.022*	0.089**	-	0.086**	0.110**	0.077**
r^2		0.52**	0.90**	0.82**	0.92**	0.94**	0.85**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† 1-92 is not included in the mean, because it was a highly germinable, non-neoteric seedlot.

‡ Values within the same row followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

§ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the germination of switchgrass seeds without stratification (G) and x is the storage temperature. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed either.

¶ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the germination of switchgrass seeds without stratification (G), x is the storage temperature, and z is the storage time. γ_0 is not listed.

Table 2.4. After-ripening and subsequent stratification response of five switchgrass seedlots as influenced by time and temperature when stored in paper bags (1994 study).

Storage time	Storage temperature	Germination with stratification (SG), by seedlots					
		1-92	1-93	12-93	1-94	13-94	Mean†
Months	°C	----------%----------					
0	-	88a‡	94a	73b	89a	71b	83
2	30	89ab	93a	77c	93a	84bc	87
	45	88b	97a	77b	89b	80b	87
4	21	90ab	95a	71c	89b	86b	86
6	5	89ab	91a	81b	89ab	85ab	87
	21	88b	98a	76c	89b	85bc	88
	30	95ab	99a	79c	90bc	89bc	91
	45	89a	95a	71b	90a	88a	87
β_1		-	0.83**	-0.23*	-	-	-
β_2		-	-0.014**	-	-	-	-
r^2		-	0.84**	0.35*	-	-	-
13	5	93a	88a	78b	90a	70b	82
	21	84a	95a	66b	86a	87a	85
	30	92ab	98a	78b	91ab	91ab	91
	45	64c	91a	36d	80b	77b	73
β_1		-	-	-0.91**	-	2.30**	1.10*
β_2		-	-	-	-	-0.042**	-0.027*
r^2		-	-	0.58**	-	0.94**	0.17**
17	5	85b	95a	70c	91ab	82bc	86
	21	84b	99a	67b	83b	85b	86
	30	83bc	98a	68c	90ab	94ab	90
	45	45bc	77a	26c	63ab	62ab	57
β_1		-0.95**	1.20**	1.60*	-0.62**	1.80**	1.30*
β_2		-	-0.032**	-0.052**	-	-0.044**	-0.039**
r^2		0.62**	0.86**	0.87**	0.52**	0.79**	0.39**
γ_1¶		1.20**	0.82**	1.20**	-	1.10**	2.00**
γ_2		-0.021**	-0.016**	-0.024**	-	-0.022**	-0.083**
γ_3		2.50**	-	2.50**	-	2.90**	0.97**
γ_4		-0.100**	-	-0.110**	-	-0.140**	-0.017**
γ_5		-0.060**	-0.009*	-0.065**	-0.022**	-	-0.034**
r^2		0.76**	0.40**	0.73**	0.43**	0.48**	0.21**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† 1-92 is not included in the mean, because it was a highly germinable, non-neoteric seedlot.

‡ Values within the same row followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

§ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the germination of switchgrass seeds that had been stratified at 5°C for 2 wk without drying (SG) and x is the storage temperature. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed either.

¶ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the germination of switchgrass seeds that had been stratified at 5°C for 2 wk without drying (SG), x is the storage temperature, and z is the storage time. γ_0 is not listed.

Table 2.5. Germination following stratification and subsequent drying of five switchgrass seedlots as influenced by time and temperature when stored in paper bags (1994 study).

Storage time	Storage temperature	Germination with stratification and drying (SDG), by seedlots					
		1-92	1-93	12-93	1-94	13-94	Mean†
Month	°C	----------%----------					
0	-	88a‡	81a	54b	27c	19c	45
2	30	86a	81a	62b	58b	41c	61
	45	86a	73ab	64bc	51c	26d	54
4	21	81a	89a	72a	78a	75a	79
6	5	93a	80b	57c	72b	54c	66
	21	90ab	94a	73c	90b	87b	87
	30	97a	95a	78b	90ab	92ab	89
	45	87b	96a	68c	86b	89b	86
β_1		-	1.20**	2.00**	1.70**	3.20**	2.00**
β_2		-	-0.017**	0.035**	-0.028**	-0.047**	-0.031**
r^2		-	0.79**	0.74**	0.81**	0.97**	0.47**
13	5	91a	75b	72b	74b	34c	64
	21	88ab	94a	62c	89ab	82b	83
	30	88a	94a	71b	92a	91a	88
	45	59c	88a	41d	78b	78b	72
β_1		1.0*	1.9**	-0.66**	2.0**	5.2**	2.5**
β_2		-0.036**	-0.032**	-	-0.038**	-0.082**	-0.045**
r^2		0.89**	0.91**	0.52**	0.87**	0.97**	0.26**
17	5	73ab	82a	56b	70ab	65ab	69
	21	82ab	91a	69b	83a	80ab	81
	30	89b	97a	78c	88b	91b	90
	45	31c	75a	15d	67ab	61b	54
β_1		3.50**	1.90**	4.10**	2.30**	3.00**	2.80**
β_2		-0.088**	-0.041**	-0.100**	-0.047**	-0.060**	-0.061**
r^2		0.90**	0.75**	0.90**	0.83**	0.81**	0.42**
γ_1¶		1.70**	1.30**	2.30**	1.50**	2.80**	1.80**
γ_2		-0.033**	-0.023**	-0.040**	-0.028**	-0.047**	-0.035**
γ_3		3.00**	2.00**	4.90**	11.00**	11.00**	6.90**
γ_4		-0.15**	-0.11**	-0.20**	-0.51**	-0.49**	-0.33**
γ_5		-0.056**	-	-0.063**	-	-	-
r^2		0.75**	0.45**	0.59**	0.91**	0.80**	0.40**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† 1-92 is not included in the mean, because it was a highly germinable, non-neoteric seedlot.

‡ Values within the same row followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

§ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the germination of switchgrass seeds that had been stratified at 5°C for 2 wk, dried at room temperature in moving air for 3 d, and rewetted (SDG) and x is the storage temperature. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed either.

¶ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the germination of switchgrass seeds that had been stratified at 5°C for 2 wk, dried in room temperature moving air for 3 d, and rewetted (SDG), x is the storage temperature, and z is the storage time. γ_0 is not listed.

Table 2.6. Dormancy reversion of five switchgrass seedlots as influenced by time and temperature when stored in paper bags (1994 study).

Storage time	Storage temperature	Reversion (R), by seedlots					
		1-92	1-93	12-93	1-94	13-94	Mean†
Months	°C	----------%----------					
0	-	11b‡	11b	19b	62a	52a	34
2	30	3c	11b	15b	34a	41a	24
	45	3c	24b	22b	37ab	54a	35
4	20	8a	6a	10a	10a	11a	9
6	5	-	10b	24ab	16ab	31a	20
	21	0a	4a	4a	-	1a	3
	30	3a	4a	4a	2a	2a	3
	45	2a	1a	2a	6a	-	3
β_1		1.20**	-0.25*	-1.70**	-	-1.30*	-1.50**
β_2		-0.017**	-	0.024*	-	-	0.023**
r^2		0.88**	0.48*	0.83**	-	0.86**	0.65**
13	5	4b	13ab	5b	16ab	36a	16
	21	4a	1a	4a	2a	5a	4
	30	3a	7a	5a	7a	1a	4
	45	8a	5a	-	2a	-	3
β_1		-	-	-	-	-1.50**	-0.40**
β_2		-	-	-	-	-	-
r^2		-	-	-	-	0.80**	0.25**
17	5	11a	12a	22a	20a	16a	17
	21	9a	11a	4a	8a	10a	9
	30	-	2a	-	6a	4a	4
	45	12a	5a	11a	4a	4a	7
β_1		-	-	-2.10*	-0.47*	-0.34**	-1.00**
β_2		-	-	-0.037*	-	-	0.015**
r^2		-	-	0.94**	0.72**	0.71**	0.39**
γ_1¶		-	-1.50*	-0.20*	-	-1.50**	-0.26**
γ_2		-	-	-	-	0.024*	-
γ_3		-	-16.00**	-2.50**	-9.60**	-6.20**	-5.40**
γ_4		-	0.800*	0.120**	0.440**	0.230**	0.240**
γ_5		0.033**	-	-	-0.028**	-	-
r^2		0.46**	0.27*	0.43**	0.93**	0.81**	0.44**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† 1-92 is not included in the mean because it was a highly germinable, non-neoteric seedlot.

‡ Values within the same row followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

§ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the dormancy reversion (R) defined as the germination of stratified seeds (SG) minus germination of seeds that were stratified, dried at room temperature in moving air for 3d, and rewetted (SDG). When SG was less than SDG, R is listed as "-". When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed either.

¶ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted for each seedlot and the mean with 1-92 excluded, where y is the dormancy reversion (R), x is the storage temperature, and z is the storage time. γ_0 is not listed.

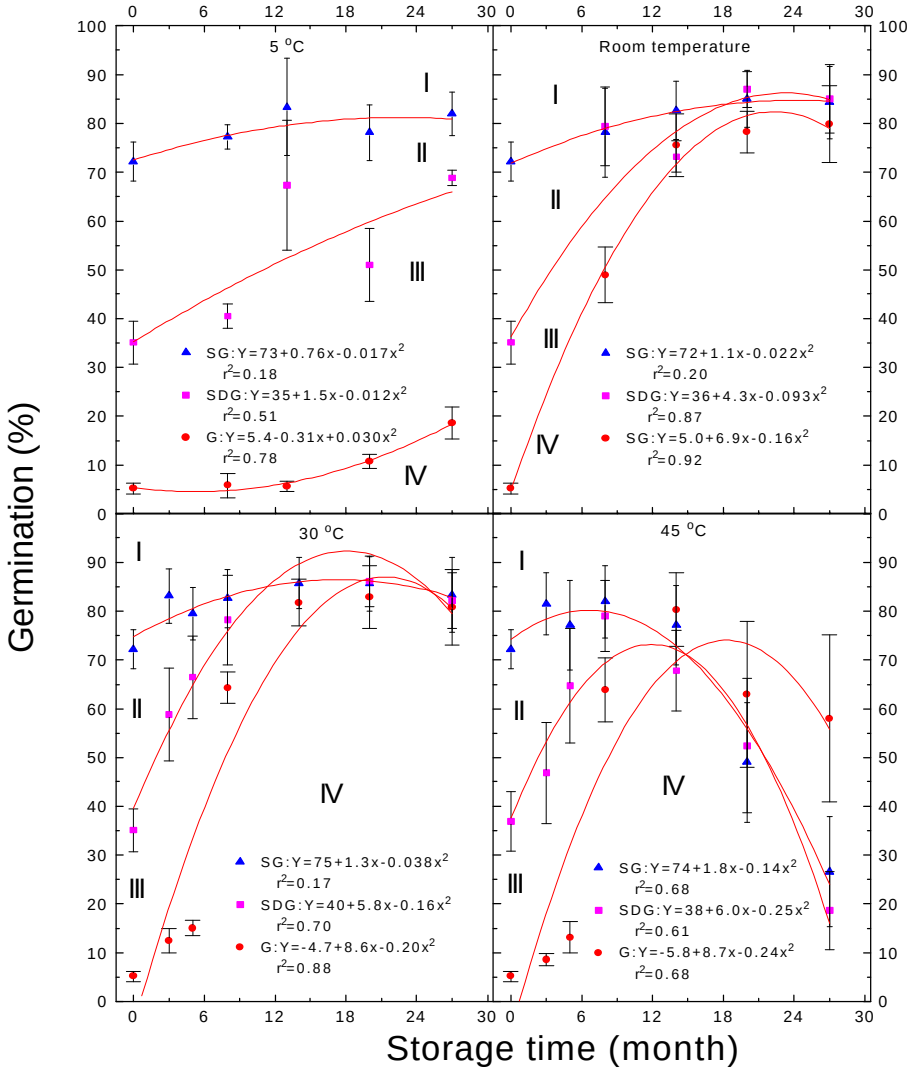

Fig. 2.1. The influence of storage temperature and time on switchgrass seed germination. The germination values include both normal and abnormal seedlings. Standard errors, indicated by vertical bars in the figure, were based on the variation among mean germination of five seedlots harvested in 1993 from Kansas, Iowa, and Virginia. ● germination without stratification (G), ▲ germination after 2 wk stratification at 5 °C(SG), ■ germination after 2 wk stratification at 5 °C plus drying for 3 d (SDG) (1993 study).

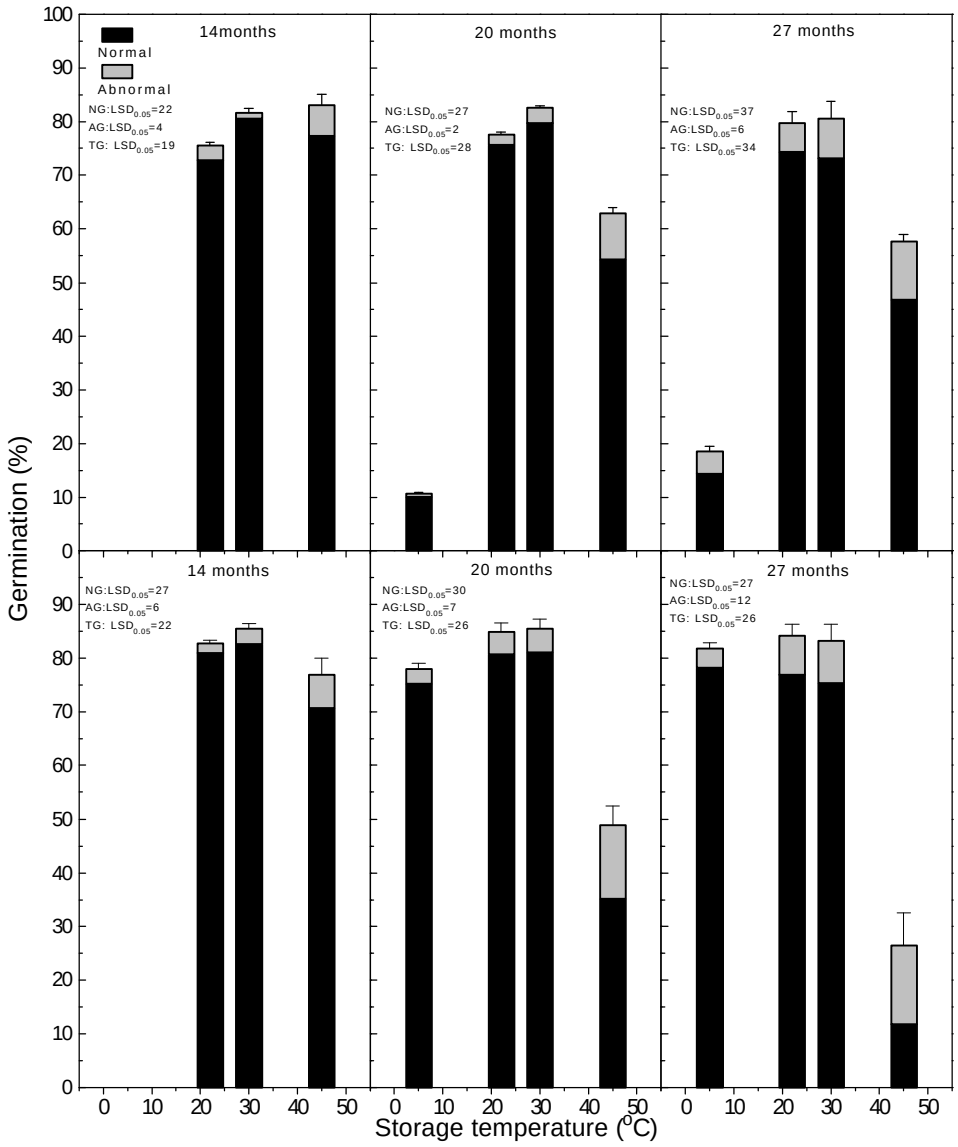

Fig 2.2. Switchgrass seed germination with normal and abnormal seedlings as influenced by storage temperature and 14, 20, and 27 mo of storage. Above: without stratification; Below: with stratification. 21°C was used to represent room temperature. No germination tests were done for seeds stored at 5°C for 14 mo since that very little dormancy was released at such a low temperature. NG:normal germination. AG: abnormal germination. TG: total germination=NG+AG. Standard errors of abnormal germinants, indicated by vertical bars, were based on the variation among mean abnormal germination of five seedlots harvested in 1993 from Virginia, Iowa, and Kansas(1993 study).

Chapter Three

After-Ripening and Aging of Switchgrass Seeds as Influenced by Seed Water Content and Storage Temperature

ABSTRACT

The influence of seed moisture content (MC) on after-ripening and aging, especially under a wide range of temperatures, requires more research. This study was conducted to find combinations of temperature and MC that might quickly increase the germinability of switchgrass (*Panicum virgatum* L.) seeds while ideally minimizing aging. The two opposing processes (after-ripening and aging) are known to be sensitive to temperature and MC in other species, but there is minimal work on their possible differential sensitivities. Seeds of "Cave-in-Rock" switchgrass harvested in 1992, 1993, and 1994 from Iowa and Virginia were stored in sealed jars at 5, 21, 30, or 45°C with MC ranging from 49 to 135 g kg^{-1}. Normal and abnormal germination were observed for seeds sampled at various times during storage. Both after-ripening and aging accelerated with increases of temperature. MC's effects were more complex, with some indication of an optimum MC whose peak value decreased with increasing temperature. As the temperature increased, after-ripening and aging differences caused by MC increased. Total germination and aging differences caused by temperature also tended to be greater at the higher MC. Storage at 60°C and 51 g kg^{-1} for about 1 mo broke most of the dormancy and resulted in acceptably low numbers of abnormal seedlings. The dormancy of switchgrass seeds may be broken by after-ripening at elevated temperatures with controlled MC. It is recommended, however, that seeds be sampled often to avoid the germination decreases caused by the concurrent accelerated aging, whose consequences can appear soon after dormancy begins to break.

INTRODUCTION

Physiological activities, to include repair mechanisms, are reduced or halted as orthodox (desiccation tolerant) seeds become dried. The vigor and ultimately the viability of a seed in dry storage is threatened by putative random aging events (Parrish and Leopold, 1978), which depend upon seed moisture level more than any other factor (Justice and Bass, 1978). After-ripening is another process (besides aging) that can occur in seed storage and that is influenced by seed moisture content. The influence of seed water content on the rate of after-ripening has probably been underestimated because of the dramatic influence of increased water content on the rate of viability loss, i.e., aging and death, in seeds (Roberts and Ellis, 1989).

It is now clear that, over a limited moisture range, there is a direct relationship in some species between the rate of after-ripening and seed moisture (Probert, 1992). Ellis et al. (1983, 1991) showed that increasing seed moisture content from 8 to 11% resulted in a 2.5-fold reduction in the storage period required to attain a given level of germination in after-ripening rice

seeds. Baskin and Baskin (1979) reported that seeds of *Draba verna* L. remained viable but did not after-ripen at 0 to 20% relative humidity (RH); a low percentage of the seeds after-ripened at 30 and 40% RH; a high percentage of the seeds after-ripened at 50 and 60% RH; and seeds rotted during storage at 70 to 100% RH.

After-ripening breaks seed dormancy at water contents too low to permit normal hydrated metabolic activity, but the activity of various physiological processes in "dry" seeds has been related to water status (Vertucci and Leopold, 1986; Leopold and Vertucci, 1989). The effect of water on seed after-ripening and aging is more directly related to water's chemical activity, of which the water potential is a direct indication. Three critical regions of water binding have been identified in seeds; each corresponds to one of the three parts of the reverse sigmoidal water sorption isotherms characteristic of desiccation-tolerant seeds (Roberts and Ellis, 1989). Based on thermodynamic principles, it has been calculated that water is highly bound in region 1 (below -350 MPa), weakly bound in region 2, and bound with negligible energy in region 3 (above -14 MPa). After-ripening of red rice occurred at moisture levels throughout region 2 (Vertucci and Leopold, 1984).

The rate of after-ripening in some dry seeds is also dependent on temperature (T), with a relatively high Q_{10} (close to 3 in some cases) (Probert, 1992; Roberts, 1965). Quail and Carter (1969) reported an interaction between T and RH for storage of *Avena ludoviciana* and *A. fatua* seeds. Relative humidity, of course, influences the equilibrium moisture content of seeds. At moderate T (25, 30, and 35°C), *Avena ludoviciana* and *A. fatua*. seeds stored at 90% RH after-ripened faster than seeds stored at 43 or 16% RH. Seeds stored at 20°C and 90% RH after-ripened more slowly than those stored at the higher T. Vertucci et al. (1994) reported that there was an optimum moisture level to reduce pea seed aging at a given T. The optimum water content for storage of pea seeds varied from 15 g kg^{-1} at 65°C to 101 g kg^{-1} at 15°C. The ubiquity and specifics of a critical or optimum water content are still a matter of some debate (Ellis et al., 1991; Smith, 1992; Vertucci and Roos, 1990, 1993a, 1993b).

The previous chapter reported that after-ripening of switchgrass seeds stored in paper envelopes was not enhanced when storage T increased from 21 to 45°C. The lack of a T response was tentatively attributed to the loss of seed moisture at higher T, which could have retarded both after-ripening and aging processes. This chapter will look more closely at the influence of T and seed moisture content (MC) on after-ripening and aging.

MATERIALS AND METHODS

Seedlot Sources

Seeds (more properly, caryopses enclosed in an extrafloral palea and lemma) of "Cave-in-Rock" switchgrass were used throughout these studies. They typically exhibited a high degree of dormancy (often less than 5% germinable) when newly harvested. Seedlots 12-93 and 13-94 were purchased from a Midwest source (Osenbaugh Grass Seeds, Lucas, IO 50151) in November 1993 and January 1995, respectively. Seedlot 1-94 was hand-collected in September 1994 from plots growing at Blacksburg, VA. Seedlot 1-92, which was high in germination, was composed of a mixture of several 1992-harvested seedlots that had been naturally after-ripened for nearly 2

yr during storage at room T. An air separator was used to clean all seedlots so that average seed weight was greater than 1.85 mg/seed (540,000 seeds/kg). All seedlots (except 1-92) were stored at 5°C following purchase or harvest. The following treatments and storage studies were begun in February 1995 and continued till June 1996.

Seed MC Adjustments and Storage

Seed moisture content (MC) of each seedlot was initially determined. Seed samples were weighed, dried in a 95°C oven for 36 hr, and reweighed. MC was determined on a dry weight basis, i.e., (Fresh Wt-Dry Wt)/Dry Wt. To achieve target MC values of 50, 75, 100, and 135 g kg^{-1}, water was either added to or removed from 200-g subsamples of each seedlot. Based on the existing and target MC and seed mass, the amount of water to be added or removed was calculated. For upward adjustments of MC, the desired amount of water was added to a small pad of tissue paper that was placed inside the container with the 200 g of seeds. By doing so and frequent stirring, no seeds imbibed excessively, and all seeds had opportunity to absorb water when the contents were mixed. To draw away water when MC had to be reduced, 200 g of seeds were placed on a small electric balance inside a vacuum chamber. The changes of seed weight detected by the balance could be viewed through a window of the chamber. The pressure inside the vacuum chamber was reduced to about 300 µm Hg. The ending point for mass of the seeds during vacuum drying was approximated by reading the balance inside the chamber and definitively established on a more sensitive balance outside the chamber.

Each moisture-adjusted seedlot was sealed in four layers of zip-closure plastic bags for 4 d to equilibrate. Seed moisture was determined again and adjusted further if necessary. After another 3 d of equilibration, the 200 g of seeds (now more or less than 200 g) for each seedlot and MC level were equally separated into samples of approximately 40 g each and placed in jars for long-term storage at various T. A total of 80, one-pint canning jars were used for seed storage [four (seedlots)*four (MC)*five (T)]. Jars were sealed with dome lids under a partial vacuum. The sealed jars were stored at 5, 21, 30, 45, or 60°C.

MC was determined for each jar at the beginning, middle, and end of storage. The MC variation between seedlots at each sampling time was small. The coefficient of variance (CV=standard deviation/mean*100%) for MC at any target level and moisture sampling time was 2.9%. Since those variations could not be used to explain the differences between seedlots, the MC reported was averaged across seedlots. A very small decline in MC (mostly less than 10 g kg^{-1}) occurred during storage. The MC reported here at each T were averaged across seedlots and MC sampling times.

Germinability and Aging Characterization

At the beginning of the storage experiment and periodically thereafter, the germinability of seedlots used in these experiments was characterized using the “ragdoll” methods described below. Sampling times for germination tests varied with MC and T. Generally, higher storage T and/or MC required more frequent sampling to observe the more rapid changes in germinability.

Upon removal from storage at various times, three subsamples of 100 seeds were

scattered on wet germination toweling, which was then rolled to form the "ragdolls". The towels weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in 17.7 by 20.3 cm plastic bags with a zip closure. Fifteen to 18 ragdolls were placed in one plastic bag. Germination tests were done by placing ragdolls (in a plastic bag) in a germinator set at 30°C. No artificial light was used in the germinator. (Preliminary studies showed that the germination percentage of switchgrass was not influenced by light). Seedlings were considered normal germinants when both coleoptile and radicle extended at least 5 mm. If the coleoptile or radicle protruded through the lemma and palea but failed to grow at least 5 mm, the seedlings were scored as abnormal germinants (AG). Total germination (TG) included both normal and abnormal seedlings. One germination count was done at 3 d to record and remove the seeds that germinated early, and a final count was made after 10 d at 30°C.

Data Analysis

Analyses of variance for all germination variables were done with PROC GLM of SAS (SAS Institute, 1988). Germination data were converted to their square root and then arcsin transformed to comply with the basic assumptions of analysis of variance; back-transformed data (sin transformation followed by square) are used for reporting. Results are reported as significant when the P-value was 0.05 or less for Tukey's Studentized Range (HSD) test. The relationships between germination and storage MC, T, and time were analyzed by regression. Indicator variables were used to compare two regression lines. The significance of indicator variables, or their interactions with other variables would suggest the difference between regression lines (Myers, 1992).

RESULTS AND DISCUSSION

Total Germination

Because of the volume, complexity, and interactions of the data from this study (Table 3.1), it has proven difficult to describe and discuss the results in a sequence that will facilitate readers' comprehension. To facilitate the process, 0-time seedlot performance will be discussed initially. Thereafter, the data will be somewhat arbitrarily divided into storage T-MC "zones", and individual seedlot performances will be compared across storage times. As the discussion progresses and builds, more and more comparisons will be made between seedlots and across storage T and MC. The readers can be guided somewhat by the subheadings used to know at what level (within which "zones") the comparisons are being made.

0-time seedlot performances

At time 0, TG of seedlot 1-92 was significantly higher than the three other seedlots, which were not significantly different from one another. This was fully expected, since 1-92 had been after-ripened for 2 yr, while the other seedlots were either recently harvested and/or had been held at 5°C since harvest. Stratification studies (data not shown) showed that the three seedlots with

low TG were highly viable. TG after 2 wk of stratification was 70 to 90% for all four seedlots. Stratified seeds of 1-94 and 13-94, however, more readily reverted to dormancy when dried after stratification (see Chapter 2). No differences were observed when comparing TG before and after MC adjustment (data not shown). The 0-time TG data presented in Table 3.1 were obtained before MC adjustments and thus are the same for all T and MC combinations.

Changes in TG for seeds stored at 5°C

The lowest storage T and MC combination was 5°C and 49 g kg^{-1}. No significant TG changes occurred between 0 and 501 d for 1-92 at this (or any) MC for 5°C. TG of 1-92 remained higher than the other seedlots during 501 d storage at 5°C at all MC tested. Between 0 and 501 d, seedlots 12-93, 1-94, and 13-94 showed linear increases in germinability. TG of 12-93 increased earlier than 1-94 and 13-94. At all storage times tested, except 0 time, TG of 12-93 was higher than 1-94 and 13-94. This difference was seen at all MC at 5°C. Separate stratification and drying studies (see Chapters 2 and 4) suggested that, at time 0, seedlot 12-93 was in a less dormant status, which was presumably related to 1 yr storage at 5°C. The time linear regression line for seedlot 12-93 was significantly different from 1-94 and 13-94 at all MC at 5°C. There was no difference between linear regression lines for 1-94 and 13-94.

At the three higher MC for 5°C, the trends of TG increases were similar to those at 49 g kg^{-1} MC. The difference between regression lines for 1-94 and 13-94 was significant at 75 g kg^{-1}, but not at 105 and 135 g kg^{-1} MC. The linear parameters for all four seedlots did not decrease or increase consistently across MC at 5°C, i.e., at low T, after-ripening was little influenced by MC. No factor was shown to be significant in the multiple regression (storage time/MC) model for 1-92 at 5°C. TG of seedlot 1-92 did not change much during nearly 1.5 yr storage at 5°C, irrespective of MC. The multiple regression model for 12-93 at 5°C showed that TG increased linearly with time and quadratically with MC. Only storage time was significant in the multiple regression model for 1-94 at 5°C. TG of 13-94 was influenced by both storage time and MC.

Changes in TG for seeds stored at 21°C

At 21°C and 50 g kg^{-1} MC, TG of 1-92 did not change significantly during 501 d of storage. In fact, neither MC nor time showed any effect on TG of the already highly germinable 1-92 during 21°C storage. TG of 1-92 remained higher than the other three seedlots throughout 501 d of 21°C storage at all MC (except when seedlot 13-94 at 501 d and 101 g kg^{-1} MC was equal to 1-92). There was a 27% increase of TG for 12-93 during the first 230 d of storage at 50 g kg^{-1}, but germination did not increase much more between 230 and 501 d. This apparent skew of the TG curve for 12-93, similar to that at 5°C and 50 g kg^{-1} MC, could possibly be explained by the seedlot's 1-yr storage history at 5°C. During or before that storage period, some after-ripening may have occurred. However, the 50 g kg^{-1} MC was perhaps not so favorable for further after-ripening at 21°C, so further TG increase was slow under this storage condition. TG of 12-93 was significantly higher than 1-94 and 13-94. The linear parameter for 12-93 was the highest among the four seedlots at 50 as well as at 78 and 101 g kg^{-1} MC for 21°C. There was no significant difference between TG for 1-94 and 13-94. Using seedlot as indicator variable, the

difference was shown to be significant between the regression lines of the four seedlots at 50 g kg^{-1} as well as other MC at 21°C.

At 21°C and 78 g kg^{-1} MC, TG of 12-93 was significantly higher than 1-94 and 13-94 at both 144 and 230 d of storage. TG of 12-93 increased 34% during the first 144 d of storage and continued to increase but more slowly after that time. At 384 d, TG of 12-93 was significantly higher than that of 13-94 but not that of 1-94. TG of 1-94 was higher than that of 13-94 at 144 and 384 d. No significant TG differences were shown between 12-93, 1-94, and 13-94 at 501 d of storage, but all three seedlots still seemed to exhibit some dormancy. The germination increases described by the regression lines of 12-93, 1-94, and 13-94 at 78 g kg^{-1} were higher than those at 50 g kg^{-1}, i.e., the higher MC was more conducive to after-ripening.

At 21°C and 101 g kg^{-1} MC, TG of 12-93 was significantly higher than 1-94 and 13-94 at 144 d of storage. No significant TG differences were shown between 12-93, 1-94, and 13-94 after 384 d of storage. When MC increased from 50 to 101 g kg^{-1}, there was accelerated after-ripening, and TG differences between seedlots (ignoring 1-92) disappeared earlier. The TG reached by 501 d of storage at 21°C increased as MC went from 50 up to 101 g kg^{-1}. The germination increases described by regression lines of 12-93, 1-94, and 13-94 at 101 g kg^{-1} were higher than those at 78 g kg^{-1}, i.e., the higher MC was, again, more favorable for after-ripening.

The trend of increasing TG (or after-ripening) as MC increased was reversed at 131 g kg^{-1} MC. The maximum germinations described by the regression lines for 12-93, 1-94, and 13-94 at 131 g kg^{-1} were shown to be less than those at 101 g kg^{-1}. TG of 12-93 was lower at 131 g kg^{-1} than at 101 g kg^{-1} after 230 d of storage. Stratification studies (Chapter 4) showed that germination of 12-93 after 2 wk of stratification was also lower at 131 g kg^{-1} than at 101 g kg^{-1} after 230 d of storage. Within the first 34 d of 21°C storage at 131g kg^{-1}, TG of 12-93 increased by 39%. Although no additional TG increase was observed for 12-93 between 34 and 230 d of storage, TG of 12-93 was significantly higher than 1-94 and 13-94 during that interval. TG of 12-93 reached the seedlot's highest observed value of 55% at 384 d and then decreased by 501 d. Stratification studies (Chapter 4) showed that germination of 12-93 after 2 wk of stratification also decreased at this time. Aging was considered as the likely cause for the decrease in germination after 501 d of storage and for the lower TG at 131 g kg^{-1} than at 101 g kg^{-1}. The lower TG of 1-94 and 13-94 at 131 g kg^{-1} compared with those at 101 g kg^{-1} could not, however, be as readily attributed to aging at the higher MC. Stratification studies (Chapter 4) showed that germination of 1-94 and 13-94 after 2 wk of stratification had not decreased as MC increased from 101 to 131 g kg^{-1}, i.e., the seeds stored at 21°C and 131 g kg^{-1} MC were less after-ripened than those stored at 101 g kg^{-1}. This is clear evidence of an optimum MC for after-ripening.

In summer annuals, low temperatures generally release dormancy, whereas high temperatures induce dormancy (Baskin and Baskin, 1977, 1987; Bouwmeester and Karssen, 1993). Our research on switchgrass (Chapter 5) shows that fully imbibed switchgrass seeds are induced into deeper dormancy when held at 21°C. In the present study, after-ripening at 21°C accelerated as MC increased from 50 to 101 g kg^{-1}. Somewhere between 101 g kg^{-1} MC and full imbibition, then, the chance of being induced into deeper dormancy by 21°C will be higher than the chance of being after-ripened. The promotive effects of chilling on the release of dormancy in apple (Vertucci and Leopold, 1986) and sitka spruce (Gosling and Rigg, 1990) can occur in seeds with a moisture content of less than 20% (fresh weight basis). While 131 g kg^{-1} MC still showed

some evidence of causing after-ripening, it was decidedly less effective than 101 g kg^{-1}. This observation is consistent with the report that there is an inverse relationship between T and MC for after-ripening of wild oat (Foley, 1994). Foley (1994) concluded that, as the after-ripening T increases, MC must decrease for maximum after-ripening (germination) to occur. It can be seen that, at least at some temperatures, after-ripening decreases as MC increases beyond some optimum. The optimum seed moisture range for after-ripening may vary with T level. Foley's explanation (1994) for the reverse relationship between T and MC is derived from moisture isotherm and water binding theory, where T and RH have opposing consequences on MC as each environmental parameter increases.

The multiple regression model across MC for 1-92 at 21°C had highly significant linear and quadratic parameters for time as well as significant linear and quadratic parameters for MC. The linear and quadratic parameters for time and MC were highly significant for seedlots 12-93, 1-94, and 13-94. Only in 1-94 was there a highly significant interaction between time and MC at 21°C. TG model across MC involved more significant factors at 21°C than at 5°C. Using T as indicator variable, the regression lines for 12-93 and 13-94 at 50 g kg^{-1} and 21°C had greater maxima than those at 49 g kg^{-1} and 5°C. The maximum germination described by the regression lines for 12-93, 1-94, and 13-94 at 78 g kg^{-1} and 21°C were greater than those at 75 g kg^{-1} and 5°C. The regression lines for all seedlots at the two higher MC at 21°C showed greater maxima than those at 5°C.

Changes in TG of seeds stored at 30°C

TG of 1-92 remained significantly higher than the other seedlots during 501 d storage at all MC tested at both 5°C and 21°C. At 30°C or above, some of the neoteric seedlots eventually equaled 1-92 in germinability at later sampling times.

At 30°C and 51 g kg^{-1} MC, TG of all seedlots increased significantly during storage. A MC of 51 g kg^{-1} resulted in 91% germination for 1-92 after 501 d of storage at 30°C. Although highly germinable at the beginning of this study, there was still a portion of the seeds in 1-92 that could be after-ripened. Under the proper storage conditions, this after-ripening increase exceeded any TG decreases caused by aging. TG of 12-93 increased more slowly after a nearly tenfold increase in the first 120 d. TG of 12-93 was generally higher than 1-94 and 13-94. TG of 12-93 at 30°C and 51 g kg^{-1} MC behaved like seeds of the same seedlots stored at 21°C and 101 g kg^{-1} MC. The germination increase described by regression lines for 1-92, 12-93, and 1-94 at 30°C and 51 g kg^{-1} MC were not significantly different from those of the same seedlots stored at 21°C and 101 g kg^{-1} MC. At the first two sampling dates, no significant differences were observed between 1-94 and 13-94. TG of 1-94 increased faster and was significantly higher than that of 13-94 between 230 and 501 d. Storage during this time at 30°C and 51 g kg^{-1} MC provided a chance for 1-94 and 13-94 to separate themselves. The germination increases described by regression lines were different among the four seedlots at 30°C and 51 g kg^{-1} MC.

At 30°C and 79 g kg^{-1} MC, TG of 1-94 and 13-94 equaled that of 12-93 after 174 d of storage. After 314 d, TG of 1-94 and 13-94 surpassed 12-93 and matched that of 1-92. Under these storage conditions, dormancy of the most neoteric seedlots was broken faster, and TG of deeply dormant seedlots more quickly equaled that of less dormant seedlots. TG of 12-93 was

significantly higher than 1-94 and 13-94 only at 120 d. No significant differences were observed between 1-94 and 13-94 throughout the storage at 30°C and 79 g kg^{-1} MC. These storage conditions revealed no differences between the two 1994 seedlots. Other pairwise comparisons between the regression lines demonstrated significant differences between the 1994 seedlots and the other two. Additionally, the regression lines for 12-93, 1-94, and 13-94 at 78 g kg^{-1} showed greater maxima than those at 50 g kg^{-1}.

At 30°C and 98 g kg^{-1} MC, TG of 1-92 did not increase significantly over time, although it appeared to be higher within the first 35 d of storage. TG of 1-92 remained significantly higher than 12-93, 1-94, and 13-94 until 314 d of storage. The highest TG observed at 30°C storage for 1-92 appeared after 111 d. Neither linear nor quadratic parameters were significant for 1-92 at 98 g kg^{-1} MC. Significant differences were indicated between the time regression lines for 1-92, 12-93, 1-94, and 13-94 at 98 g kg^{-1} MC. The regression lines for 1-92, 12-93, and 1-94 at 98 g kg^{-1} were not significantly different from those at 79 g kg^{-1} MC. The regression lines for 12-93, 1-94, and 13-94 at 98 g kg^{-1} had greater maxima than those at 51 g kg^{-1} MC. TG of 1-94 and 13-94 at 120 and 230 d (presumably at 174 d also) were significantly higher at 79 than at 51 g kg^{-1} MC. By contrast, TG of 1-94 and 13-94 at 174 d was significantly lower at 98 than at 79 g kg^{-1} MC. This difference was consistent with the trend of TG changes for these two 1994 seedlots over storage time at 51, 79, and 98 g kg^{-1} MC. It was also consistent with the earlier observed trend of an MC optimum for after-ripening at 21°C. The viability of 1-94 and 13-94 after 174 d of storage at 98 g kg^{-1} MC was shown by stratification (Chapter 4) to be at least 91 and 89%, respectively. These seedlots were still exhibiting moderately high levels of dormancy, and that dormancy was being broken more quickly at 79 than at 98 g kg^{-1} MC. The optimum MC for after-ripening appeared to shift downward as storage T increased. Further research is needed on this TG decrease that happened as MC increased from 79 to 98 g kg^{-1} at 30°C or from 101 to 131 g kg^{-1} at 21°C.

Within the first 24 d at 30°C and 131 g kg^{-1} MC, TG of 1-92 appeared to increase slightly. TG of 1-92 was significantly higher than that of other seedlots through 111 d of storage. The subsequent steady decrease of 1-92 TG at 30°C and 131 g kg^{-1} MC was reflected in the negative linear parameter. A TG decrease was also more evident for 12-93 and perhaps even earlier than for 1-92. The sharp TG decrease after 68 d of storage resulted from the dying of most 12-93 seeds before release from dormancy, which was reflected in the fact that TG of 12-93 never equaled that of 1-92. TG of 1-94 equaled that of 1-92 at 174 d (when 1-92 was already declining) and then started to decrease quickly. No TG decrease was observed for seedlot 13-94 at 30°C and 131 g kg^{-1} MC. It may be significant that the seedlot with an earlier TG increase would also have an earlier TG decrease. At 30°C and 131 g kg^{-1} MC, some seeds will probably die before being released from dormancy. When switchgrass seeds are stored at the warmer conditions to hasten after-ripening, MC needs to be monitored to reduce the complicating and adverse influence of aging. The time regression lines of the four seedlots at 30°C and 131 g kg^{-1} MC were all significantly different from one another. The after-ripening and aging processes described by the regression line for each seedlot at 131 g kg^{-1} were shown to be faster than at 98 g kg^{-1}.

The regression lines for 1-92 at 30°C and 51 or 131 g kg^{-1} MC had higher maxima than those at 21°C and 50 or 131g kg^{-1} MC. All the other seedlots had regression lines at 30°C

significantly different from those at 21°C with similar MC. The multiple regression model across MC for all seedlots had larger parameters at 30°C than at 21°C. The multiple regression model across MC for each seedlot indicates that the after-ripening and aging processes were influenced significantly by MC, storage time, and/or their interaction.

Changes in TG of seeds stored at 45°C

At 45°C and 51 g kg^{-1} MC, TG of seedlots 12-93 and 13-94 reached 70% within the first 144 d of storage. TG of 13-94 increased more slowly but attained 83% by 310 d of storage. Seedlot 1-92 trended toward a TG decrease after 144 d of storage under these conditions. The regression line of each seedlot was shown to be significantly different from the line at 30°C and 51 g kg^{-1} MC; the seeds were after-ripening and, in some cases, aging more quickly at the higher T. The two older seedlots appeared more likely to show signs of aging and seed death.

After 130 d of storage at 45°C and 71 g kg^{-1} MC, TG of 1-92 reached the highest value observed for it in any treatment. TG of 12-93 was significantly higher than 1-94 and 13-94 at 24 and 44 d of storage. TG of 1-94 and 13-94 exceeded 70% after 91 d of storage and increased steadily till 203 d of storage. No difference was indicated between the regression lines for 1-94 and 13-94. Pairwise comparisons between the regression lines demonstrated significant differences between the other seedlots. The after-ripening and aging processes described by regression lines for all seedlots at 71 g kg^{-1} were faster than those at 48 g kg^{-1}.

At 45°C and 104 g kg^{-1} MC, TG of seedlots 12-93 and 1-94 started to decrease before reaching 50%. Such a high MC at 45°C was not suitable for after-ripening. TG of 1-92 was significantly higher than others until 41 d of storage. TG of 13-94 increased through 77 d of storage, however, with more increase of abnormal seedlings (to be discussed later). The regression lines of the four seedlots were all different from one another. The regression lines for each seedlot at 104 g kg^{-1} described after-ripening and aging processes that were faster and with lower maxima than their counterparts at 71 g kg^{-1}. The highest MC for 45°C (130 g kg^{-1}) was not included in the table, since all seeds died in a very short storage time.

The regression lines for all seedlots at 45°C changed faster than those at 30°C with similar MC. The multiple regression model across MC for each seedlot indicated that the after-ripening and aging processes were influenced significantly by MC, storage time, and/or their interaction.

Changes in TG of seeds stored at 60°C

It was quite interesting to observe that, at 60°C and 51 g kg^{-1} MC, TG of 1-94 and 13-94 reached over 70% after only 23 to 42 d of storage. TG of 1-92 remained above 80% through 23 d of storage but fell thereafter. TG of 12-93 increased to be 63% within the first 23 d of storage and then decreased very quickly. Seed dormancy could be released within a relatively short time of after-ripening at this T and MC. The time required for each seedlot to achieve the highest TG varied. Continuous sampling for germination at about 1-wk intervals would be necessary to determine the critical time to end the enhanced after-ripening before TG decreases to unacceptable levels due to aging. The regression lines of the four seedlots were shown to be

different at 51 g kg^{-1} MC, which was also true for all the other MC at 60°C.

TG of 1-92 fell slightly to 61% by 21 d of storage at 77 g kg^{-1} MC and 60°C. The highest TG attained by the other seedlots at this MC and T was lower than 61%, and TG decreased for the neoteric seedlots faster than for 1-92. Many seeds apparently died before being released from dormancy under these storage conditions. The same phenomena were more evident at 104 g kg^{-1} MC and 60°C. The highest MC for 60°C (130 g kg^{-1}) was not included in the table, since all seeds died in a very short storage time. At temperatures as high as 60°C, only seeds stored at very low MC could survive and thus had a chance to be after-ripened to be germinable. The regression lines for all seedlots were different at all MC when stored at 60°C. Although it was evident that, under these harsh conditions, dormancy was not a shield against aging, it could also be true under less harmful condition. TG of highly germinable seedlot 1-92 was not shown to decrease as early as 12-93 at any T-MC combination. For unknown reasons, 1-92 appeared to be less susceptible to aging at 60°C and at higher MC than the other seedlots. Even at several lower T-MC combinations, seedlot 1-92 seemed less sensitive to aging. The regression lines for all seedlots at 60°C changed faster and with lower maxima than those at 45°C with similar MC. The multiple regression model across MC for all seedlot 60°C had highly significant linear and quadratic parameters for time, MC, and the interaction between them.

With all the variations between seedlots and the possibility of describing the relationships between the factors involved in a complicated model, the experimental results with the ranges of T and MC tested may best be summarized as follows: 1) TG of neoteric seedlots increased for some time as T and MC increased; the length of the effective after-ripening period decreased as T and MC increased, i.e., after-ripening would increase slowly for a long time at low T and MC and quickly for a short time at higher T and MC; the higher the T and MC, the shorter the time before after-ripening effects were overtaken by aging and death. 2) TG increased as T and storage time increased, when seeds were stored at lower MC; the "safe" MC (at which no seed death was detected) decreased as T and storage time increase, i.e., after-ripening would increase with MC at low T and early times and decrease with MC at higher T and later times; the higher the T and the longer the storage time, the lower the MC that could be tolerated before seed death was evident. 3) TG increased as MC and storage time increased when seeds were held below a certain T; the "safe" T (at which no seed death was observed) decreased as MC and storage time increased, i.e., after-ripening would increase with T at low MC and early times but decrease with T at higher MC and later times; the higher the MC and the longer the storage time, the lower the T that would result in seed death. 4) There usually was a transitional area where no significant trend of TG change with T, MC, and storage time was observed. 5) Most significantly perhaps, there was an optimum MC for after-ripening, and the optimum shifted downward as T increased.

Abnormal Germination

Up to this point, we have been examining total germination (TG), i.e., normal (NG) plus abnormal germinants (AG), because we wanted to observe shifts in dormancy. An AG is, after all, not dormant. The presence of AG would seem to be a good indication of the aging process, however, because AG appears even before seeds begin to die and TG falls. If one uses elevated T or MC treaments to enhance after-ripening, the degree of aging (as indicated by AG) along with a

fall in the TG should suggest the ending point for the treatments. Again, due to the volume, complexity, and interdependencies of the AG data from the study, the presentation of the AG data will follow the general discussion pattern for TG.

Time 0 seedlot differences in AG

At time 0, AG was very small for all seedlots (Table 3.2). Seedlots 1-94 and 13-94 had no AG, which may be related to their very low TG at time 0 as well as their newness. Tukey's analysis showed that the AG of 1-92 was significantly higher than 1-94 and 13-94. The newer (1994) seedlots were apparently more vigorous (less aged) and therefore produced fewer abnormal germinants.

Changes in AG of seeds stored at 5°C

AG at 5°C was quite small for all storage MC and times (Table 3.2). One thing perhaps worthy of mention is that, even under the lowest MC and T, some abnormal seedlings were still observed. There were also trends toward increasing AG at longer times and higher MC. It was not possible to determine if these abnormal seedlings were caused by aging or perhaps by a partial release of the axis from dormancy (where the root/radicle might be more or less germinable than the shoot/coleoptile).

Changes in AG of seeds stored at 21°C

AG at 21°C was also quite small for all MC and times (Table 3.2). The decrease of germinability of 12-93 after 501 d at 131 g kg^{-1} (Table 3.1) was not reflected in a change of AG. If the decline of 12-93 was due to aging, seeds apparently died before their declining state was detected as AG.

Changes in AG of seeds stored at 30°C

At 30°C and 50 g kg^{-1} MC, no significant linear or quadratic changes for AG were observed for any seedlot through 501 d of storage. Switchgrass seeds after-ripened slowly without clear evidence of aging detectible by AG under this condition. AG was low compared with TG (Table 3.1, 3.2).

At 30°C and 78 g kg^{-1} MC, linear increases for AG were seen in 12-93 and 1-94. AG for 13-94 had a highly significant linear parameter and significant quadratic parameter. No significant change for AG was observed for seedlot 1-92. Significant differences for AG between seedlots were seen only at 314 d, when 12-93 was higher. The decrease of AG for 12-93 between 314 and 384 d coincided with a small decrease in TG during the same time (Table 3.1). AG was, again, low compared with TG.

At 30°C and 98 g kg^{-1} MC, AG for all seedlots increased through 501 d. No differences in AG were observed between seedlots during storage for up to 174 d. Most abnormal seedlings appeared after 174 d. For seedlots 1-92 and 12-93, the increase in AG between 230 and 314 d

was much higher than for TG. The net decrease in NG indicated that 230 d or sooner should have been the ending point for storage of 1-92 and 12-93 for the purposes of after-ripening. Between 230 and 501 d, 12-93 had a higher AG than 13-94. For seedlots 1-94 and 13-94, the increase in TG was greater than AG through 501 d of storage (Table 3.1, 3.2), i.e., the net number of "good" seedings continued to increase as more after-ripening occurred and aging was not yet felt as AG.

The increases of AG for 1-92, 1-94, and 13-94 were shown to be faster at 131 g kg^{-1} MC than at 98 g kg^{-1} MC. The decrease of TG and increase of AG after 68 d at 30°C and 131 g kg^{-1} MC resulted in a dramatic increase of the proportion of abnormal seedlings for 1-92. The AG for 12-93 was, however, less at 131 g kg^{-1} MC than at 98 g kg^{-1} MC. This was consistent with the dramatic decrease of TG for 12-93 at 131 g kg^{-1} MC. When seeds aged quickly, most seeds were not detected as producing abnormal seedlings before dying. No significant differences in AG were observed between seedlots during storage up to 111 d at 30°C and 131 g kg^{-1}MC. The decrease of TG and increase of AG for 1-94 happened after 174 d of storage, 106 d later than 1-92. Although TG kept on increasing through 501 d for 13-94, the increase of AG resulted in a decrease of normal germinants from 36 to 27% between 230 and 314 d of storage (Table 3.1, 3.2). Storage conditions of 30°C and 131 g kg^{-1} MC were not optimal for switchgrass after-ripening.

The multiple regression model across MC for each seedlot indicated that AG was influenced significantly by MC, storage time, and their interaction (Table 3.2).

Changes in AG of seeds stored at 45°C

At 45°C and 48 g kg^{-1} MC, the linear increases of AG over time were significant for three seedlots, but not 1-92. No significant differences in AG were shown among seedlots through 384 d of storage. By 501 d, AG increased while TG decreased for 12-93 (Table 3.1), and 20 out of 51 seedlings were abnormal. The increase of AG for 1-94 and 13-94 between 384 and 501 d was occurring at the same time as a slight decrease in TG, so normal germinants declined decidedly in the second year of storage at this T-MC combination, i.e., aging was taking a toll.

At 45°C and 71 g kg^{-1} MC, both linear and quadratic parameters were significant for all seedlots except 1-92. The increase of AG was so dramatic after 91 d that most germinable seeds were abnormal by 203 d. For seedlot 12-93, all germinable seeds were abnormal at 203 d. No significant differences were seen among seedlots up to 44 d of storage at 71 g kg^{-1} MC. At 91 and 130 d, AG of 12-93 was higher than the other seedlots. At 152 d, AG of 12-93 was higher than 1-94. AG of 12-93 was lower than other seedlots at 203 d, but that was only because all of its other seeds appeared to be dead. Storage for 91 d would have been an appropriate ending time for this MC-T combination, since TG for each seedlot was close to or above 70% and AG was mostly low (except for 12-93) (Table 3.1, 3.2).

The change of AG at 45°C and 104 g kg^{-1} MC was somewhat similar to but more rapid than that which occurred at 30°C and 131 g kg^{-1} MC. Between 24 and 59 d, AG for 1-92 increase by 39% while TG was decreasing by 28 % (Table 3.1, 3.2). The decrease in AG for 1-92 after 59 d paralleled the decrease of TG at the same time. The AG for 12-93 was much lower at 104 than at 71 g kg^{-1} MC, which was due to rapid death of seeds. The 20% increase of AG for 1-

94 between 41 and 59 d occurred at the same time as a slight decrease in TG. Between 41 and 77 d, the increase of AG was 39%, while the increase of TG was only 26%. Storage conditions of 45°C and 104 g kg^{-1} MC were not good for switchgrass after-ripening, because aging and death appeared to occur more rapidly than after-ripening could break dormancy.

The multiple regression model across MC for each seedlot indicated that AG was influenced significantly by MC, storage time, and their interaction (Table 3.2).

Changes in AG of seeds stored at 60°C

It was interesting to observe that AG at 60°C and 51 g kg^{-1} MC did not increase much until after 23 d, when TG for all seedlots was above 51% (Table 3.1, 3.2). AG increased dramatically for all seedlots except 13-93 between 23 and 42 d, when TG decreased. AG increased by 5% for 13-94 between 23 and 42 d, while TG increased by 28%. The 22% increase of AG for 13-94 between 42 and 48 d occurred at the same time as a 6% decrease in TG, i.e., the seedlot was rapidly aging. A slight decrease of TG was often accompanied by a greater decrease of AG.

At 60°C and 77 g kg^{-1} MC, the highest AG observed for 12-93 and 1-94 was below 10% due to the rapid death of seeds. The greatest increase of AG for 1-92 and 13-94 happened between 13 and 21 d. At 60°C and 104 g kg^{-1} MC, all seedlots died so quickly that very few live seeds (even abnormal) were observed. Aging increased with each increment of T and MC (after-ripening does not). The death of seeds stored at 60°C and 135 kg^{-1} happened within a few hours and was not reported here.

The multiple regression model across MC for each seedlot indicated that AG was influenced significantly by MC, storage time, and their interaction (Table 3.2). As the temperature increased, the aging process dramatically accelerated, which can be seen from the increase of the parameters of fitted multiple regression equation.

SUMMARY AND CONCLUSIONS

Germination of dormant switchgrass seeds increased and then decreased as T, MC, and/or storage time increased. The germination increase was caused by after-ripening, and the decrease caused by aging. After-ripening at lower T and MC was slow, so dormancy break by after-ripening increased as T and MC increased. After-ripening, however, was observed to be slowed when MC increased beyond 101 and 79 g kg^{-1} at 21 and 30°C, respectively. It was suggested that this slow down of after-ripening at relatively higher MC could be related to the phenomenon that imbibed switchgrass seeds stored at warmer conditions will deepen in dormancy. Probably the efficiency of after-ripening would decrease as seed MC was in the transitional range between after-ripening and dormancy deepening. This decreased efficiency of after-ripening at high MC was not observed at 5°C, probably because after-ripening was too slow to allow MC to differentiate. This decreased efficiency of after-ripening at high MC was observed at neither 45 nor 60°C, probably because aging masked or negated after-ripening at high MC. Aging, reflected both by decreases in germinability and increases in abnormal seedlings, increased continuously as T, MC, and storage time increased. When seeds aged quickly, the increase of abnormal seedlings

may not be seen before seed death results in a decrease of the total germinability. The increase or decrease of germinability was determined by the combined influence of after-ripening and aging at specific storage conditions and times. Germinability was also influenced by the response of specific seedlots, probably related to the seed's history.

Storage at 5°C was safe for seeds but only reduced seed dormancy very slowly, even with MC up to 135 g kg^{-1}. Storage at 21°C was also safe for seeds, but it took nearly 1.5 yr for seeds to have TG of more than 60%. Storage at 30°C and 51 g kg^{-1} caused little detectable aging but also broke dormancy slowly. Both aging and after-ripening occurred too rapidly to allow a high TG to develop at 30°C and 131 g kg^{-1}. Medium MC (79 to 98 g kg^{-1}) at 30°C gave an acceptable AG but still required more than 0.5 yr to produce nearly 60% germinability. Seeds stored in paper bags typically have MC around 75 g kg^{-1} when equilibrated at 30°C (Table 2.2). Storage at 45°C and 48 g kg^{-1} produced little detectable aging but also required about 5 mo to allow germinability to reach nearly 50%. Seeds stored in paper bags have MC around 45 g kg^{-1} when equilibrated at 45°C. The lowered MC (at 45°C) explains why considerably longer times were needed for after-ripening even when T was increased for seeds in paper bags (see Chapter 2). Storage at 45°C and 71 g kg^{-1} for 3 mo broke most of the dormancy and had acceptable numbers of abnormal seedlings. Aging was too rapid to allow a high TG to be observed at 45°C and 104 g kg^{-1}. Storage at 60°C and 51 g kg^{-1} for about 1 mo broke most of the dormancy and resulted in acceptably low numbers of abnormal seedlings. Both aging and after-ripening were too rapid to allow a high TG to be observed at 60°C and 77 or 104 g kg^{-1}. Since both after-ripening and aging can occur rapidly at high T and MC, both T and MC must be well controlled, and seeds need to be sampled frequently to stop at the highest TG point. Seedlot differences need also to be watched closely under enhanced conditions for after-ripening and aging.

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Table 3.1. Total germination (TG=normal plus abnormal germinants) of four switchgrass seedlots as influenced by storage temperature (T), mean seed moisture content (MC) during storage, and storage time.

Storage			TG, by seedlot			
T	MC	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
5	49	0	77a†	4b	3b	3b
		230	76a	14b	6c	6c
		384	76a	22b	8c	6c
		501	76a	29a	9c	9c
β_1‡			-	0.49**	0.12**	0.10**
β_2			-	-	-	-
r^2			-	0.83**	0.69**	0.50*
	75	0	77a	4b	3b	3b
		230	82a	15b	5c	7c
		384	81a	21b	5c	9c
		501	83a	26b	6c	12c
β_1			-	0.42**	-	0.17**
β_2			-	-	-	-
r^2			-	0.94**	-	0.79**
	105	0	77a	4b	3b	3b
		230	75a	19b	6c	6c
		384	77a	23b	11c	13c
		501	73a	31b	11c	14c
β_1			-	0.50**	0.18**	0.23**
β_2			-	-	-	-
r^2			-	0.92**	0.60**	0.78**
	135	0	77a	4b	3b	3b
		174	80a	34b	6c	6c
		230	77a	20b	4c	7c
		384	75a	24b	11c	7c
		501	71a	35b	12c	10c
β_1			-	0.47*	0.19**	0.12**
β_2			-	-	-	-
r^2			-	0.41**	0.64**	0.64**
γ_1§			-	0.47**	0.14**	0.16**
γ_2			-	-	-	-
γ_3			-	-	-	2.2**
γ_4			-	0.50**	-	-1.1**
γ_5			-	-	-	-
r^2			-	0.69**	0.47**	0.69**

Table to be continued on the next page

Table 3.1. continued

Storage			TG, by seedlot			
T	MC	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
21	50	0	77a	4b	3b	3b
		230	82a	31b	6c	8c
		384	82a	37b	13c	15c
		501	79a	33b	11c	23c
β_1			-	1.7**	0.18**	0.39**
β_2			-	-0.22**	-	-
r^2			-	0.89**	0.66**	0.74**
	78	0	77a	4b	3b	3b
		144	87a	38b	18c	9d
		230	85a	39b	22c	17c
		384	83a	48b	38b	23c
		501	83a	50b	47b	41b
β_1			-	2.1**	0.87**	0.72**
β_2			-	-0.26**	-	-
r^2			-	0.91**	0.97**	0.87**
	101	0	77a	4b	3b	3b
		144	83a	40b	16c	26d
		230	82a	48b	21c	36b
		384	88a	62b	50b	60b
		501	82a	63b	59b	72ab
β_1			-	2.6**	1.2**	1.6**
β_2			-	-0.30**	-	-
r^2			-	0.97**	0.94**	0.96**
	131	0	77a	4b	3b	3b
		34	82a	43b	13c	10c
		230	80a	43b	18c	12c
		384	83a	55b	44bc	26c
		501	77a	48b	49b	31c
β_1			-	0.65*	-	-
β_2			-	-	0.19**	0.11**
r^2			-	0.49*	0.92**	0.77**
γ_1			0.42**	2.0**	-	0.74**
γ_2			0.10**	-0.25**	-	-
γ_3			2.9*	9.2**	13**	18.6**
γ_4			-1.6*	-4.0**	-6.9**	-9.5**
γ_5			-	-	0.87**	-
r^2			0.31**	0.82**	0.87**	0.70**

Table to be continued on the next page

Table 3.1. continued

Storage			TG, by seedlot			
T	M	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
30	51	0	77a	4b	3b	3b
		120	86a	38b	12c	7c
		230	83a	48b	31c	10d
		314	85a	50b	38b	17c
		384	89a	59b	45c	26d
		501	91a	61b	58b	40c
β_1			0.22**	2.4**	1.2**	-
β_2			-	-0.26**	-	0.14**
r^2			0.46**	0.91**	0.95**	0.93**
	79	0	77a	4b	3b	3b
		120	85a	53b	34c	30c
		174	88a	63b	60b	57b
		230	86a	69b	63b	65b
		314	86ab	74b	77ab	87a
		384	83a	72b	84a	81a
β_1			0.87**	4.6**	3.6**	3.7**
β_2			-0.19*	-0.76**	-0.38**	-0.39**
r^2			0.41**	0.97**	0.96**	0.94**
	98	0	77a	4b	3b	3b
		35	85a	41b	18bc	12c
		68	80a	44b	18c	9d
		111	88a	56b	42b	15c
		174	84a	61b	40c	31c
		230	82a	65b	65b	52c
		314	84a	70b	71b	63b
		384	82a	67b	85a	71b
		501	81a	66b	86a	79a
β_1			-	3.2**	3.1**	2.4**
β_2			-	-0.46**	-0.29**	-0.15**
r^2			-	0.82**	0.95**	0.96**
	131	0	77a	4b	3b	3b
		24	86a	35b	8c	6c
		68	79a	38b	20c	8d
		111	71a	30c	49b	21c
		174	56a	24c	54a	36b
		230	50a	12b	43a	51a
		314	29b	5c	17b	55a
β_1			-1.7**	1.8*	6.0**	2.2**
β_2			-	-0.78**	-1.7**	-
r^2			0.85**	0.51**	0.88**	0.93**
γ_1			1.4**	4.4**	2.5**	1.2*
γ_2			-	-0.36**	-0.23**	-0.16**
γ_3			17**	33**	24**	21**
γ_4			-8.8**	-17**	-12**	-11**
γ_5			-1.8**	-2.1**	-	1.2**
r^2			0.67**	0.77**	0.79**	0.82**

Table to be continued on the next page

Table 3.1. continued

Storage			TG, by seedlot			
T	MC	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
45	48	0	77a	4b	3b	3b
		24	83a	27b	8c	10c
		68	85a	48b	30c	10d
		144	86a	70b	75ab	46c
		314	85a	69b	84a	83a
		384	79a	72a	87a	83a
		501	76a	51b	81a	82a
β_1			0.59*	4.3**	5.1**	4.0**
β_2			-0.14**	-0.73**	-0.71**	-0.45**
r^2			0.38**	0.79**	0.95**	0.97**
	71	0	77a	4b	3b	3b
		14	81a	18b	8b	9b
		24	88a	38b	7d	11c
		44	84a	54b	26c	18c
		91	88a	68b	75b	74b
		130	92a	63b	80ab	86ab
		152	85a	55b	84a	84a
		203	76b	44c	88a	86a
β_1			2.1**	10**	10**	10**
β_2			-1.1**	-4.5**	-2.7**	-2.8**
r^2			0.38**	0.86**	0.96**	0.95**
	104	0	77a	4b	3b	3b
		24	83a	23c	38b	17c
		30	76a	36b	41b	29b
		41	69a	21d	49b	36c
		59	55a	11b	47a	54a
		77	31b	2c	24b	62a
β_1			3.6*	11**	21**	8.1**
β_2			-13**	-16**	-22**	-
r^2			0.94**	0.73*	0.88**	0.95**
γ_1			4.8**	9.9**	6.1**	5.9**
γ_2			-0.27*	-0.94**	-0.93**	-0.81**
γ_3			28**	33**	19**	26**
γ_4			-18**	-21**	-10**	-14**
γ_5			-7.2**	-9.5**	-	-
r^2			0.58**	0.69**	0.86**	0.89**
60	51	0	77a	4b	3b	3b
		7	81a	39b	14c	6c
		9	81a	36b	11c	3d
		14	81a	51b	38bc	23c
		17	82a	53b	44c	30d
		23	83a	63bc	77ab	51c
		42	68a	22c	45b	79a
		48	42b	8c	0d	73a
β_1			11**	44**	54**	17**
β_2			-35**	-94**	-103**	-
r^2			0.92**	0.96**	0.75**	0.91**

Table to be continued on the next page

Table 3.1. continued

Storage			TG, by seedlot			
T	M	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
60	77	0	77a	4b	3b	3b
		3	83a	27b	10c	8c
		7	85a	47b	37bc	23c
		9	84a	41b	28b	39b
		13	81a	24c	8c	53b
		16	79a	18c	0d	49b
		21	61a	0c	0c	24b
β_1			20*	59**	35**	73**
β_2			-130**	-310**	-200**	-270**
R^2			0.59**	0.75**	0.50**	0.77**
	104	0	77a	4b	3b	3b
		1	70a	11b	2b	11b
		2	58a	0b	0b	5b
		2.3	51a	0c	0c	3b
		3	37a	0b	0b	0b
		4.2	24a	0b	0b	0b
β_1			-130**	-19**	-9.8**	-17*
β_2			-	-	-	-
r^2			0.91**	0.39**	0.45**	0.32**
γ_1			57**	110**	130**	48**
γ_2			-43**	-100**	-110**	-24*
γ_3			53**	39**	36**	39**
γ_4			-35**	-24**	-21**	-23**
γ_5			-81**	-130**	-150**	-36*
r^2			0.68**	0.84**	0.75**	0.83**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Values within the same row for each storage temperature, seed moisture content, and time followed by the same lower case letters were not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

‡ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seed moisture level under each storage temperature, where y was the TG and x was the storage time. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Numbers listed in table for β_1 and β_2 have a factor of 10^{-1} and 10^{-3}, respectively. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed.

§ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted to each temperature level, where y was the TG, x was the storage time, and z was seed moisture. Numbers listed in table for γ_1, γ_2, γ_4, and γ_5 have a factor of 10^{-1}, 10^{-3}, 10^{-1}, and 10^{-2} respectively. γ_0 is not listed.

Table 3.2. Abnormal germination (AG) of four switchgrass seedlots as influenced by storage temperature (T), mean seed moisture content (MC) during storage, and storage time.

Storage			AG, by seedlot			
T	MC	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
5	49	0	2a	1ab	0b	0b
		230	5a	2ab	1b	2ab
		384	3a	2a	0a	0a
		501	5a	4a	1a	3a
β_1‡			-	-	-	-
β_2			-	-	-	-
r^2			-	-	-	-
	75	0	2a	1ab	0b	0b
		230	5a	2a	1a	0a
		384	4a	2a	0a	0a
		501	4a	3a	2a	0a
β_1			-	-	-	-
β_2			-	-	-	-
r^2			-	-	-	-
	105	0	2a	1ab	0b	0b
		230	5a	3ab	1b	2ab
		384	2a	2a	1a	2a
		501	3a	4a	3a	2a
β_1			-	0.52*	-	0.49**
β_2			-	-	-	-
r^2			-	0.35*	-	0.77**
	135	0	2a	1ab	0b	0b
		174	2a	3a	0a	2a
		230	4a	3a	0b	2a
		384	4a	5a	0a	1a
		501	10a	6a	1a	4a
β_1			-	0.98**	-	0.68**
β_2			-	-	-	-
r^2			-	0.72**	-	0.44**
γ_1§			0.20**	-	-	-
γ_2			-	-	-	-
γ_3			-	-	-	-
γ_4						
γ_5			-	0.063**	0.020*	0. 052**
r^2			-	0.35**	0.10*	0.35**

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Table 3.2. continued

Storage			AG, by seedlot			
T	MC	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
21	50	0	2a	1ab	0b	0b
		230	4a	4a	2a	1a
		384	3a	3a	1a	0a
		501	1a	0a	1a	0a
β_1			-	2.7*	-	-
β_2			-	-0.53*	-	-
r^2			-	0.42*	-	-
	78	0	2a	1ab	0b	0b
		144	1ab	7a	1b	2ab
		230	5a	4a	1a	2a
		384	4a	3a	3a	1a
		501	2a	3a	1a	1a
β_1			-	2.6*	-	-
β_2			-	-0.50*	-	-
r^2			-	0.31*	-	-
	101	0	2a	1ab	0b	0b
		144	2a	1a	1a	0a
		230	5a	5a	1a	2a
		384	4a	3a	1a	2a
		501	2a	3a	3a	1a
β_1			-	-	0.49*	-
β_2			-	-	-	-
r^2			-	-	0.35*	-
	131	0	2a	1ab	0b	0b
		34	4a	3a	0a	2a
		230	4a	4a	2a	3a
		384	4a	3a	1a	1a
		501	4a	2a	2a	0a
β_1			-	-	-	-
β_2			-	-	-	-
r^2			-	-	-	-
γ_1			1.1*	2.4*	0.30*	0.94**
γ_2			-0.21*	-0.44*	-	-0.14*
γ_3			-	-	-	-
γ_4			-	-	-	-
γ_5			-	-	-	-
r^2			0.088	0.25**	0.10*	0.17**

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Table 3.2. continued

Storage			AG, by seedlot			
T	M	T	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
30	51	0	2a	1ab	0b	0b
		120	6a	3a	1a	0a
		230	5a	4a	4a	1a
		314	4ab	9a	3b	3ab
		384	1a	2a	1a	0a
		501	2a	5a	3a	2a
β_1			-	-	-	-
β_2			-	-	-	-
r^2			-	-	-	-
	79	0	2a	1ab	0b	0b
		120	6a	7a	3a	3a
		174	2a	4a	1a	3a
		230	3a	3a	2a	2a
		314	3b	11a	5ab	3b
		384	5a	6a	5a	3a
β_1			-	1.7*	1.2**	2.2**
β_2			-	-	-	-0.45*
r^2			-	0.24*	-	0.44**
	98	0	2a	1ab	0b	0b
		35	4a	4a	1a	1a
		68	1a	0a	1a	0a
		111	3a	1a	1a	0a
		174	2a	5a	2a	2a
		230	6ab	8a	3ab	2b
		314	19a	24a	9b	6b
		384	9ab	18a	11ab	5b
		501	14ab	23a	9b	9b
β_1			2.7**	5.1**	2.8**	1.9**
β_2			-	-	-	-
r^2			0.52**	0.73**	0.72**	0.71**
	131	0	2a	1ab	0b	0b
		24	0a	0a	0a	0a
		68	6a	3a	1a	1a
		111	4a	3a	1a	1a
		174	8a	7a	7a	3b
		230	22a	6b	17a	15ab
		314	21ab	3c	11b	28a
β_1			7.2**	6.0**	4.9**	-4.5**
β_2			-	-1.5**	-	4.2**
r^2			0.73**	0.67**	0.32**	0.97**
γ_1			-5.3**	-	-2.4**	-5.0**
γ_2			-	-	-	-
γ_3			-44**	37**	-21*	-49**
γ_4			21**	-22**	10*	24**
γ_5			0.86**	0.33**	0.50**	0.81**
r^2			0.60**	0.52**	0.63**	0.67**

Table to be continued on the next page

Table 3.2. continued

Storage			AG, by seedlot			
T	MC	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
45	48	0	2a	1ab	0b	0b
		24	0a	0a	0a	1a
		68	2a	2a	1a	1a
		144	3a	4a	2a	3a
		314	6a	7a	2a	4a
		384	2a	16a	4a	2a
		501	4b	20a	11ab	9ab
β_1			-	4.5*	-	1.2*
β_2			-	-	0.38**	-
r^2			-	0.40**	0.80**	0.58**
	71	0	2a	1ab	0b	0b
		14	2a	0a	0a	0a
		24	1a	2a	0a	0a
		44	2a	0a	1a	0a
		91	7b	27a	10b	5b
		130	18b	42a	16b	16b
		152	42ab	50a	21b	28ab
		203	62a	44b	62a	65a
β_1			-	44**	-14**	-18**
β_2			15**	-7.8*	20**	24**
r^2			0.93**	0.89**	0.92**	0.98**
	104	0	2a	1ab	0b	0b
		24	3a	0b	1ab	0b
		30	20a	8a	10a	4a
		41	22a	10a	11a	7a
		59	42a	9c	31ab	27b
		77	27b	2c	20b	46a
β_1			43**	33*	36**	-19*
β_2			-	-38*	-	105**
r^2			0.62**	0.27*	0.63**	0.99**
γ_1			-44**	-23**	-36**	-54**
γ_2			-	-	-	-
γ_3			-	180**	-9.2*	-120*
γ_4			-	-120**	-	63*
γ_5			9.6**	5.9**	-8.0**	11**
r^2			0.77**	0.63**	0.71**	0.82**
60	51	0	2a	1ab	0b	0b
		7	1a	1a	0a	0a
		9	2a	0a	0a	0a
		14	6a	6a	1a	2a
		17	0a	3a	0a	0a
		23	0c	7a	5ab	2bc
		42	29ab	19b	38a	7c
		48	25a	8b	0c	29a
β_1			-	29**	42**	-
β_2			120**	-	-	190**
r^2			0.78**	0.60**	0.28**	0.77**

Table to be continued on the next page

Table 3.2. continued

Storage			AG, by seedlot			
T	M	Time	1-92	12-93	1-94	13-94
°C	g kg^{-1}	days	----------%----------			
60	77	0	2a	1ab	0b	0b
		3	0a	1a	0a	0a
		7	1a	4a	3a	0a
		9	3a	7a	8a	2a
		13	8a	9a	3a	9a
		16	23a	6a	0a	14a
		21	43a	0c	0c	20b
β_1			-	150*	93*	110*
β_2			1000**	660*	460*	-
r^2			0.73**	0.30*	0.30*	0.64**
	104	0	2a	1ab	0b	0b
		1	0a	0a	0a	0a
		2	0a	0a	0a	0a
		2.3	6a	0b	0b	0b
		3	3a	0b	0b	0b
		4.2	11a	0b	0b	0b
β_1			-	-23*	-	-
β_2			5000*	-	-	-
r^2			0.51**	0.30*	-	-
γ_1			-270**	28**	35**	-240**
γ_2			130*	-	-	190**
γ_3			-	-	-	-9.8*
γ_4			-	-	-	-
γ_5			53**	-	-	39**
r^2			0.67**	0.43**	0.28**	0.74**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Values within the same row for each storage temperature, seed moisture content, and time followed by the same lower case letters were not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's test and then back transformed for reporting.

‡ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seed moisture level under each storage temperature, where y was the AG and x was the storage time. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Numbers listed in table for β_1 and β_2 have a factor of 10^{-1} and 10^{-3}, respectively. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed.

§ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted to each temperature level, where y was the TG, x was the storage time, and z was seed moisture. Numbers listed in table for γ_1, γ_2, γ_4, and γ_5 have a factor of 10^{-1}, 10^{-3}, 10^{-1}, and 10^{-2} respectively. γ_0 is not listed.

Chapter Four

Post-Stratification Dormancy Reversion of Switchgrass Seeds as Influenced by Stratification and After-Ripening

ABSTRACT

The germinability of neoteric seedlots of switchgrass (*Panicum virgatum* L.) often increases by many-fold to 80% or more upon stratification if the seeds are moved directly to germination without drying. However, germinability may fall by half or more if the seeds are dried between stratification and germination. The high germinability can again be restored by further stratification. It is of both scientific and practical value to further define this phenomenon of "reversion". Reversion as influenced by post-stratification drying, prolonged stratification, and after-ripening was investigated using seeds of "Cave-in-Rock" switchgrass harvested in 1992, 1993, and 1994 from the Midwest and Virginia. Switchgrass seeds stratified for 2 wk were dried to different moisture contents to observe the influence of the extent of post-stratification drying on reversion. Continuous and interrupted stratifications of 2 to 8 wk were imposed to observe their influence on dormancy reversion. The influence of after-ripening on reversion was also observed following storage in jars at 5, 21, 30, or 45°C with seed moisture contents ranging from 49 to 135 g kg^{-1}. Basal germination, germination following stratification, and germination with stratification and drying were observed for seeds sampled at various times during storage. During post-stratification drying, the degree of dormancy reversion increased as the desiccation proceeded. Revertibility decreased as stratification or after-ripening time increased. Stratification and after-ripening worked additively to release switchgrass seeds from dormancy and reversion. The reversion phenomenon strongly suggests that seeds vary in their degree or depth of dormancy and germinability, i.e., that the seed exists in some state along a continuum of dormancy-germinability and that it can be moved toward either greater germinability or greater dormancy. The reversion phenomenon provides a unique way to observe the degree of seed dormancy and the plasticity of dormancy-germinability. Increased after-ripening (by manipulating storage temperature and seed moisture content) reduced revertibility, i.e., moved seed further toward a fully germinable state. Stratification for 6 wk will prevent dormancy reversion of stratified seeds when dried post-stratification. After-ripening for as long as 1 yr can also minimize the dormancy reversion of switchgrass seeds.

INTRODUCTION

Seed dormancy in switchgrass can be broken by various treatments to include exposing seeds to a relative short period of wet, cool conditions (usually referred to as stratification). The effects of stratification on seed dormancy in many species have been well covered in a number of reviews and general texts (Lewak and Rudnicki, 1977; Nikolaeva, 1977; Bewley and Black, 1982; Mayer and Poljakoff-Mayber, 1989).

In farm practice, dormancy of neoteric switchgrass seedlots might be overcome by

stratification. However, wet seeds need to be dried before planting. We have found that this drying can sometimes lead to a reduction in germinability (but not viability). We define the decrease in germinability of switchgrass seeds when dried following stratification as " reversion". This decrease in germinability is not a result of death caused by drying, because reverted seeds will become germinable when stratified again. We have noted that the reversion to dormancy during drying seems less likely to happen if seeds are stratified for extended periods or if they are after-ripened sufficiently. The reversion to dormancy during drying seems to occur when seeds have been "marginally" treated to break dormancy, i.e., stratified for minimal periods (2 wk or less) or after-ripened under less than optimal conditions of time, moisture, or temperature (see Chapter 3).

We previously described some aspects of dormancy reversion of switchgrass seeds during a storage study (Chapter 2), but that study had no control of seed moisture during storage. In this study, we will describe more completely the phenomenology of dormancy reversion. We are interested in the effects of post-stratification drying extent, stratification times, and after-ripening on the reversion of switchgrass seeds.

MATERIALS AND METHODS

Seedlot Sources

Seeds of "Cave-in-Rock" switchgrass were used throughout these studies. They typically exhibited a high degree of dormancy (usually less than 5% germinable) when newly harvested. Seedlots 19-92 and 26-92 were purchased in February of 1993, 12-93 in November of 1993, and 13-94 in January 1995 all from a Midwest source (Osenbaugh Grass Seeds, Lucas, IO 50151). Seedlot 1-94 was hand-collected in September 1994 from plots growing at Blacksburg, VA. Seedlot 1-92, which was highly germinable, was composed of 1992-harvested seeds that had been naturally after-ripened during storage for nearly 2 yr at room temperature. An air separator was used to clean all seedlots so that average seed weight was greater than 1.85 mg/seed (540,000 seeds/kg). All seedlots (except 1-92) were stored at 5°C after purchase or harvest.

Dormancy Reversion as Influenced by Extent of Post-Stratification Drying

Two post-stratification drying studies were performed. Seedlot 19-92 was stored at 5°C for about 1 yr before post-stratification drying for 0 to 12 h in November of 1993. Seedlot 13-94, which had been stored at 5°C and 50 g kg^{-1} for about 2 yr, was stratified and dried for 0 to 72 hr in March 1997. (From previous storage studies, it was known that seeds stored under cool and dry conditions have very little of their dormancy broken and are thus essentially neoteric). About 25 g of seeds from each seedlot were placed in cloth bags and immersed in distilled water at 5°C for 24 h. After draining off the excess water (around 400 g kg^{-1} MC was retained by seeds), the seeds were stratified at 5°C for 2 wk in the cloth bags inside of sealed plastic bags. Seeds were then spread out and dried for varying periods in an open, shallow container at ambient (laboratory) temperature and humidity. After 6 hr drying by natural evaporation, moving air was used to speed up the drying in both 1993 and 1997 studies. Seeds were frequently stirred to ensure the evenness of moisture loss. Care was thus taken to ensure the periodic samples for

moisture determinations and germination tests were of uniform dryness and representative. Small samples were taken at intervals for gravimetric determination of seed moisture content. Moisture content (MC) was determined on a dry weight basis, i.e., (Fresh Wt-Dry Wt)/Dry Wt, after drying for 24 hr at 95°C. Three samples of 100 seeds each were also taken at each drying interval for germination testing. These partially to fully dehydrated seeds were scattered on wet germination toweling which was then rolled to form "ragdolls". The 23 by 31 cm towel weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in a 17.7 by 20.3 cm plastic bag with a zip closure. Fifteen to 18 ragdolls were placed in one plastic bag for germination at 30°C. No artificial light was used in the germinator or during stratification. (Preliminary studies showed that the germination of switchgrass was not influenced by light). One germination count was done at 3 d to observe and remove the early germinated seeds, and a final count was taken after 10 d at 30°C. Seedlings were considered normal germinants when both coleoptile and radicle were extended at least 5 mm. If the coleoptile or radicle protruded through the lemma and testa but failed to grow at least 5 mm, the seedling was scored as an abnormal germinant. Germination values presented in this chapter include both abnormal and normal seedlings unless otherwise specified.

Dormancy Reversion as Influenced by Length and Interruption of Stratification

Eighty-gram samples of seedlots 1-92, 19-92, and 26-92 were placed in cloth bags and submerged in water at 5°C for 24 hr. After draining off the excess water, the seeds (at about 400 g kg^{-1} MC) were stratified for varying times at 5°C in the cloth bags placed inside of sealed plastic bags. After 2, 4, 6, or 8 wk of this "bulk" stratification, some subsamples were removed and placed in 100-seed ragdolls which went directly to a germinator at 30°C. Other subsamples removed after 2, 4, 6, or 8 wk of bulk stratification at 5°C were spread out and dried at room temperature in moving air for 3 d before being placed in ragdolls for immediate rewetting and germination testing at 30°C. (Seeds reached equilibrium with air and had less than 100 g kg^{-1} MC after 3 d drying at room temperature in moving air). Still other bulk-stratified seeds were dried at room temperature in moving air for 3 d, placed in ragdolls, rewetted, and further stratified at 10°C for 2 more wk before germination testing at 30°C. In one more variation, after 2 or 4 wk of bulk stratification at 5°C, some seeds were dried and rewetted as above for 2 more wk of stratification at 5°C, which resulted in a 5°C stratification time totaling 4 or 6 wk but with a dehydration interruption. At the end of the interrupted 5°C stratification periods, the ragdolls were either tested for germination immediately, dried in moving air for 3 d and then tested for germination, or dried for 3 d followed by 2 wk at 10°C and then tested for germination. The initial, continuous stratification temperature was set at 5°C, because we have observed some seeds germinate while being held at 10°C for longer times.

Dormancy Reversion as Influenced by After-ripening at Different Temperatures and Seed Moisture Contents

Samples of seedlots 1-92, 12-93, 1-94, and 13-94 were adjusted in moisture content (MC) (see Chapter 3) to 48, 71, and 104 g kg^{-1} and stored in sealed jars at 45°C. At the beginning of the 45°C storage and periodically thereafter, the influence of post-stratification drying on

germinability (see below) was observed. The sampling intervals were shorter at the higher MC, since earlier studies (Chapter 3) showed seeds would after-ripen (and age) more quickly at these MC. For seeds stored at 104 and 71 g kg^{-1}, germination observations stopped after 77 and 203 d, respectively, due to the significant increase of abnormal seedlings (around 50% of the germinants were abnormal).

Dormancy reversion was also examined in the above four seedlots after they had been stored in sealed jars for about 1 yr at 5, 21, 30, or 45°C at MC up to 104 g kg^{-1}. After storage for 384 d, the influence of post-stratification drying on germinability was observed. The single, longer sampling period was suitable for all MC tested at these lower temperatures. The higher MC levels were avoided at the higher temperatures.

Germination testing procedures were the same for seeds sampled at all times, MC, and temperatures. Basal germination (G) was obtained by placing ragdolls with 100 seeds at 30°C for 10 days. Germination following stratification (SG) was determined by placing seeds in ragdolls at 5°C for 14 d before germination at 30°C for 10 d. Germination following stratification and drying (SDG) involved stratifying seeds in ragdolls at 5°C for 14 d, drying seeds while in the ragdolls at room-temperature in moving air for 3 d, and then germination at 30°C for 10 d after rewetting. Drying the ragdolls for 3 d resulted in seed MC of about 100 g kg^{-1}.

Data Analysis

Analyses of variance for all germination variables were done with PROC GLM of SAS (SAS Institute, 1988). Germination data were converted to their square root and then arcsin transformed to comply with the basic assumptions of analysis of variance; back-transformed data (sin transformation followed by square) are used for reporting. Results are reported as significant when the P-value is 0.05 or less for Tukey's Studentized Range (HSD) test. The relationships between germination and storage MC, T, and time were analyzed by regression method.

RESULTS AND DISCUSSION

Dormancy Reversion as Influenced by Extent of Post-stratification Drying

Seedlot 19-92 was 4% germinable without stratification. After 2 wk of stratification at 5°C, its germination (SG) increased to 84%. Seedlot 13-94 had a similar low germinability; its initial germination of 8% increased to 65% after 2 wk of stratification. Both seedlots showed a steady decrease of germinability (or increase in reversion) as MC of the seeds decreased during post-stratification drying (Fig. 4.1). It is perhaps significant that no definite MC "threshold" was observed for germinability decrease, i.e., reversion became evident with very little dehydration. Perhaps there was a threshold at which individual seeds reverted to dormancy, but differences in threshhold values and maybe differences in drying status allowed some seeds to revert to dormancy when the MC of seeds in total had just started to decrease. The lowest germination (corresponding to the lowest MC) of 13-94 seeds was very close to the germination and MC levels of seeds in ragdolls that were stratified at 5°C for 2 wk and dried at room temperature for 3 d (data not shown here) (our standard procedure for observing SDG, see Chapters 2 and 3).

Dormancy Reversion as Influenced by Length and Interruption of Stratification

Stratification at 5°C for 2 wk increased the germination of neoteric seedlots 19-92 and 26-92 from 5 to 81% and 9 to 70%, respectively (Table 4.1). Drying significantly reduced their germinability to 45 and 33%, respectively. Further stratification (2 more wk at 10°C) following drying brought their germination back to 83% and 93%, respectively. Obviously, then, the germination decrease caused by drying was not due to the death of seeds. Stratification for 4 wk at 5°C greatly reduced or eliminated the reversion caused by post-stratification drying of seedlot 19-92. Significant reversion was still observed for seedlot 26-92 after 4 wk of stratification. Continued stratification of 6 and 8 wk brought germination to more than 90% for seedlots 19-92 and 26-92. Drying after 6 or 8 wk of stratification did not significantly reduce the germinability of stratified seeds. For all seedlots, germinability (with no treatment or with drying following stratification) increased quadratically over stratification time. Dormancy reversion could be reduced by prolonged stratification. Interrupted stratification for a total of 4 wk (2 wk at 5°C, 3 d drying, 2 wk further at 5°C) surprisingly broke significantly more dormancy for 26-92 than 4 wk of continuous stratification at 5°C. After 6 wk of stratification, germination with or without an interspersed drying was not different (6 wk continuous versus 4+2 interrupted). In farm practice, continuous stratification for 6 wk would be recommended to maximize germination (minimize reversion) of switchgrass seeds.

Germination of seedlot 1-92, which had been largely after-ripened before this study began, was high even without stratification, but it was increased slightly by 2 wk of stratification. No reversion was observed when seeds stratified for 2 wk were dried. No significant germination differences were shown among drying, drying plus further stratification, and further stratification treatments during continuous or interrupted stratification. All of these treatments resulted in a relatively stable 75 to 85% germination.

Dormancy Reversion as Influenced by After-ripening

Dormancy reversion of four switchgrass seedlots as influenced by seed moisture content when stored at 45°C

In each of Figures 4.2 through 4.5, the white area above the SG curve yet below 100% represents seeds which were either dead or not made germinable by stratification at 5°C for 2 wk. The darkest shaded area between the curves for SG and SDG reveals those seeds that revert into dormant status if 2 wk of stratification is followed by drying. The intermediate shading between SDG and G represents those seeds that become germinable when stratified for 2 wk and remain germinable after post-stratification drying. The lightly shaded area below G shows the seeds that are inherently nondormant or have been after-ripened sufficiently to become germinable. The area between SG and G (intermediate and darkest shading) indicates the portion of seeds whose dormancy is broken when stratified for 2 wk.

The after-ripening time required for all seedlots to minimize dormancy reversion decreased as MC increased to 104 g kg^{-1}. Seedlot 13-94 seemed to be the most deeply dormant of the four seedlots tested in this particular trial. It had the lowest SG and the highest dormancy reversion at time 0 (Fig 4.2). At 104 g kg^{-1} MC, SG for 13-94 reached a maximum at 41 d and then

decreased, but it reached the peak more slowly than other seedlots at the same MC. G and SDG did not decrease until after 77 d storage at 104 g kg^{-1} MC. At 71 g kg^{-1} MC, G continued to increase through about 120 d. SG and SDG peaked earlier but did not decrease much through 150 d. At 48 g kg^{-1} MC, both SG and SDG reached their maximum of about 90% after 68 d of storage, when G was only 10%. The maximum value of G was reached much later. The lowest MC presumably provided the least after-ripening and presented the longest and greatest differential between SG and SDG, i.e., the most revertibility.

Reversion was seen in seedlot 1-94 at 104 g kg^{-1} MC through 66 d of after-ripening at 45°C (Fig. 4.3). The early decrease of SG and SDG of 1-94 indicated that seeds held under such high-temperature, high-moisture conditions were aged very quickly. SG and SDG probably decreased earlier than G because seeds aged by the harsh storage conditions would be further stressed by stratification and drying. Seedlot 1-94 was very similar to 13-94 in change of G, SG, and SDG at 71 kg^{-1} MC. The increase of G, SG, and SDG was again slower at 48 than at 71 g kg^{-1} MC. After 24 d of after-ripening at 45°C and 48 g kg^{-1} MC, nearly half of the stratifiable seeds reverted to a dormant state if dried after stratification. No reversion was observed after 68 d of after-ripening at this lowest MC tested, while at the same time, only 30% of the seeds germinated without stratification (G). The increase of G after 68 d was faster for 1-94 than for 13-94, although both reached their maximum at about the same time.

The reversion at 0 time was much less for 12-93 (Fig.4.4) than for 1-94 at time 0. Reversion was no longer evident in seedlot 12-93 after the first 10 d of after-ripening at 104 g kg^{-1} MC and 45°C. The highest G was observed between 24 and 30 d. When G was maximum, there was a large portion of seeds still dormant. As they were perhaps being released from dormancy, other seeds were losing viability quickly so that all parameters fell after 41 d. At 71 g kg^{-1} MC, G, SG, and SDG of 12-93 increased in a parallel way through 44 d of after-ripening. While SG and SDG started to decrease after 44 d, G continued to increase for another 15 d and then started to level off and decrease. The decrease of G for 12-93 at times when there were a considerable number of seeds still dormant means some potentially after-ripened seeds must have died before being released from dormancy. The increase of G, SG, and SDG was slower at 48 than at 71 g kg^{-1} MC. Although reversion disappeared after about 180 d of after-ripening at 48 g kg^{-1} MC, a small portion of the seeds remained dormant until 480 d of storage. The maximum G, SG, and SDG achieved during storage was smallest for 12-93 among all four seedlots examined.

Seedlot 1-92, which had been naturally after-ripened on the lab bench for 2 yr, showed evidence of additional after-ripening but minimal reversion when held at 45°C and 101 g kg^{-1} MC. Its G and SDG increased in parallel in the first 24 d and decreased quickly thereafter (Fig.4.5). No reversion of 101 g kg^{-1} MC was observed for 1-92 seeds; on the contrary, SG was actually lower than SDG at all but one sampling date. At 71 g kg^{-1} MC, G and SDG of seedlot 1-92 reached maxima by 14 d. Again no reversion was observed; SG was slightly higher than SDG only at 91 d. A very small portion of the seeds of 1-92 showed evidence of dormancy reversion between 30 and 180 d storage at 48 g kg^{-1} MC. The highly germinable 1-92 seedlot still had a small portion of seeds in dormancy, and the variations in degree of dormancy could be observed as dormancy was released slowly.

When considering the three more neoteric seedlots in this study, we can make some generalizations. During the early stages of after-ripening at all three MC, the increase of SDG was faster than G, which suggests that more seeds were moving from a deeply dormant state to a

semi-dormant (revertible) category than from a semi-dormant to a fully germinable (nonrevertible) category. After-ripening during this period released a portion of the seeds from dormancy, while reducing the depth of dormancy of other seeds. After-ripening appeared to first reduce the degree of dormancy (increase SG and SDG), and then the amount of dormant seeds as usually defined (increase G). It would appear also that the amount of after-ripening and/or stratification determined the degree of dormancy; i.e., either after-ripening or stratification (or the combination) can move the seeds' physiological state along the continuum from deeply dormant, to semi-dormant, to fully germinable.

Dormancy reversion of four switchgrass seedlots at time 0 and after 384 d storage at various temperatures and seed moisture contents.

Dormancy reversion of seeds at time 0. At time 0, G was significantly lower than SG and SDG for all four seedlots, which meant that all seedlots had at least some dormant seeds (Table 4.2). All seedlots but 1-92 showed significant differences between SG and SDG, which meant that some seeds were in deeper dormancy than others, i.e., were less fully responsive to stratification and could revert to dormancy during post-stratification drying. Most seeds of 1-94 and 13-94 would revert to dormancy if they were stratified for 2 wk and dried.

Dormancy reversion of seeds stored at 5°C for 384 d. SDG of both 13-94 and 1-94 was significantly lower than SG but higher than G at all MC (Table 4.2). With about 1 yr of after-ripening at 5°C, most seeds of 13-94 and 1-94 were still dormant. Some of those dormant seeds within each seedlot were either still ungerminable after 2 wk of stratification at 5°C, or they reverted to dormancy during drying following stratification. The differences between SG and SDG were no longer significant for 12-93 and 1-92 at all MC, i.e., there was no reversion after about 1 yr of after-ripening at 5°C. With the increase of MC, SG and SDG for seedlots 13-94 and 1-94 as well as G for 13-94 showed significant change. As shown by the significance of the polynomial parameters, the more deeply dormant seedlots were more responsive than less dormant ones to differences in MC under low temperature storage.

Dormancy reversion of seeds stored at 21°C for 384 d. Depending on MC and seedlot, G and SDG could be two to five fold higher at 21°C that at 5°C after 1 yr of storage (Table 4.2). Only SDG of 13-94 and 1-94 stored at 50 g kg^{-1} was significantly lower than SG but higher than G, i.e., reversion disappeared for most treatments after 1 yr of after-ripening at 21°C. However, G was significantly lower than SG for both 1-94 and 13-94 after 1 yr at any MC. G and SG were equal (not significantly different) for 12-93 and 1-92 when stored at the two higher MC, i.e., they were fully after-ripened and/or fully germinable, thus not responding to stratification or post-stratification drying. After 1 yr of after-ripening at 21°C, some dormancy still existed in two seedlots, while reversion had disappeared. This suggests more seeds had been released from deep dormancy (were no longer revertible after stratification) than had been totally released from dormancy. Put in another way, we can speculate that 1 yr of after-ripening under these conditions plus stratification brought the seeds to a more fully germinable state on the continuum between fully dormant and fully germinable; one where they must still be stratified for 2 wk to become germinable but where 2 wk of stratification will make them so germinable that they will not revert if dried post-stratification.

Seedlots 13-94, 12-93, and 1-92 showed a decrease in G, SG, and SDG as MC increased

from 101 to 131 g kg^{-1}. This decline likely resulted from seeds aging faster when stored at the higher MC. However, the great decrease of G for 13-94 was difficult to explain in the same way, since both SG and SDG did not decrease. More likely, slow down of after-ripening at high MC could be the reason (see Chapter 3 on optimum MC for after-ripening). We may be seeing a temperature-moisture combination where for some seedlots, increased MC does not accelerate after-ripening.

Dormancy reversion of seeds stored at 30°C for 384 d. No differences were seen between SG and SDG for all seedlots and MC except 1-94 stored at 79 g kg^{-1}. The SDG change over MC was significant only for seedlot 12-93 (Table 4.2), and there the changes seemed slight and uncertain. After 1 yr of after-ripening at 30°C, reversion disappeared even from the 13-94 and 1-94 seedlots that had been deeply dormant as indicated by G and reversion. G was still significantly lower than SG, however, for 13-94 at all MC, 1-94 and 12-93 at the two lower MC, and 1-92 at 79 g kg^{-1}. An increase of G and SG followed by a decrease was observed for most seedlots. The difference in G between 21 and 30°C increased as MC increased from low to medium level but decreased as MC increased from medium to high level. After-ripening for 1 yr at 30°C did not release all seeds from dormancy, especially at low MC.

Dormancy reversion of seeds stored at 45°C for 384 d. No differences were observed between S, SG, and SDG of seedlots 13-94 and 1-94. After-ripening at 48 g kg^{-1} and 45°C for 384 d removed all detectable evidence of seed dormancy for these two seedlots that had been deeply dormant. Seedlots 12-93 and 1-92 showed a significantly lower SG than SDG. Since this difference was consistent with data from other sampling dates (not reported here), it was proposed that the drying and rewetting treatments for SDG may give more chance for seeds to repair damage caused by aging. This would be a sort of seed-priming effect that can improve the germination of some aged seeds (Pill et al., 1994).

General trends of germination change across MC and T after about 1 yr storage. Parameters that were significant in multiple regressions varied with seedlots at 384 d of storage. Both T and MC influenced S, SG, and SDG; but the degree of influence varied with seedlots because of the differences in the pace of dormancy release and aging. Dormancy reversion disappeared from most seedlots at most storage treatments by 1 yr of after-ripening. Storage at 5°C or at 49 g kg^{-1} and 21°C, however, did not reduce dormancy reversion of 1994-harvested seedlots as effectively as higher T and MC. Dormancy had not been fully broken in most seedlots at most storage treatments during the 1 yr of after-ripening, i.e., most seeds were less deeply dormant but were not unqualifiably germinable yet. There was some residual dormancy tendency and/or the seeds were not yet in a fully germinable state. At the higher T, the change of G over MC tended to be greater. G, SG, and SDG increased first and then declined as T and MC went up. Increased T and MC enhanced dormancy break, but more aging was also caused by higher T and MC.

Sometimes it may not be enough to observe G and SG to know a seedlot's position on the dormancy-germinability continuum. SDG may be more revealing. Seed scientists tend to regard germination as an on/off process; a germinable seed is by definition "on", and a dormant seeds is "off" (Amen, 1968). Thus, all physiological and biological measurements about germination are assumed to be related to "on-ness". In dormant seeds, most physiological processes are thought of as inactive or at least covert. There are few ways to monitor physiological parameters that might be causing shifts in dormancy (toward the "on" state), since there are few ways to quantify

dormancy itself except in an absolute yes/no sense. The reversion phenomenon strongly suggests there is a "degree of dormancy", and it may serve as a marker for shifts in the state or depth of dormancy. It has been suggested that "deep dormancy" requires longer stratification to overcome (Bewley and Black, 1982; Mason et al., 1995). SG, SDG, and G all can reflect the degree of seed dormancy. Reversion appears to provides a unique means to tell how deep is the dormancy for a seedlot at a particular time.

The mechanism by which dormancy reversion occurs is not known. It seems reasonable that the germination decrease caused by drying was not due to physiological damage. Rather the reversion seemed to be turned on progressively by added increments of drying, as though drying itself caused the dormancy shift. There could be one or several common mechanisms or chemicals involved in dormancy release (or intensification) by after-ripening, stratification, or physical and chemical treatment, as well as dormancy induction by post-stratification drying or by warm-wet conditions (Chapter 5).

SUMMARY AND CONCLUSIONS

The germinability of not fully after-ripened, 2-wk stratified switchgrass seeds can decrease as they dry following stratification. We have coined the term "reversion" to describe this phenomenon. The degree of dormancy reversion increases as the desiccation increases. The reverted seeds can be made germinable again by further stratification. Stratification for 2 wk is not sufficient in farm practice to break dormancy of neoteric seedlots, since a considerable portion of the seeds will revert to dormancy when dried for mechanical planting. Stratification for 6 wk is recommended. Revertibility decreases as after-ripening increases. After-ripening appears to first reduce the degree or depth of stratifiable dormancy (increasing SDG), and then to overcome the remaining residual dormancy (increasing G). The reduction of reversion occurs more quickly under higher T and MC, which also accelerate the after-ripening process.

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Table 4.1. Germination percentage and time regression factors of switchgrass seedlots after continuous and interrupted stratification.

Seedlot	Treatment following 5°C stratification	Germination, by weeks of stratification at 5°C									
		Continuous								Interrupted	
		0	2	4	6	8	β_1†	β_2	r^2	2+2	4+2
		----------%----------								--------%--------	
19-92	None	5b‡	81a	81aA§	91aA	93a	29.0**	-2.5**	0.88	90abA	92aA
	Drying¶	5b	45b	76aA	81bA	84a	23.9**	-1.8**	0.97	83bA	85aA
	Drying+S_f#	88a	83a	90aA	85abB	88a	-	-	0.01	91abA	90aA
26-92	None	9b	70b	74bB	90aA	90a	24.7**	-1.9**	0.92	89aA	89aA
	Drying	9b	33c	55cB	93aA	83a	17.9**	-0.94*	0.93	90aA	90aA
	Drying+S_f	80a	93a	87aA	88aA	90a	-	-	0.10	90aA	88aA
1-92	None	66a	83a	83aA	83aA	84a	6.5**	-0.59*	0.60	87aA	87aA
	Drying	66a	82a	86aA	83aA	81a	7.6**	-0.76*	0.58	81aA	85aA
	Drying+S_f	76a	78a	73aA	82aA	81a	-	-	0.10	81aA	85aB

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted to each treatment, where y is the germination and x is the weeks of stratification. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Original data were transformed for Tukey's analysis and then back transformed for reporting. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed.

‡ Values within the same column and seedlot followed by the same lowercase letter are not significantly different at the 5% level based upon Tukey's multiple range test.

§ Values within the same row followed by the same uppercase letter indicate that the germination after 4 or 6 wk of continuous stratification is not significantly different from 2+2 or 4+2 wk of interrupted stratification at the 5% level based upon Tukey's multiple range test.

¶ Seeds were dried at room temperature in moving air for 3 d. With 0 wk of stratification, seeds were already dry and thus no drying treatment was done.

Further stratification for 2 wk at 10°C.

Table 4.2. Dormancy reversion of switchgrass seeds before and after about one year after-ripening at various temperatures (T) and seed moisture contents (MC).

			Germination, by seedlot											
Storage			13-94			1-94			12-93			1-92		
Time	T	MC	G	SG	SDG	G	SG	SDG	G	SG	SDG	G	SG	SDG
Day	°C	g kg^{-1}	----------%----------											
0	-	-	3c†	71a	21b	3c	89a	28b	4c	73a	54b	77b	88a	88a
384	5	49	6c	48a	26b	8c	80a	34b	22b	69a	60a	76b	92a	86a
		75	9c	63a	37b	5c	81a	53b	21b	66a	64a	81b	91a	92a
		105	13c	66a	40b	11c	90a	61b	23b	70a	67a	77b	90a	90a
		135	7c	67a	37b	11c	84a	53b	24b	74a	63a	75b	89a	90a
	β_1‡		0.55*	1.0*	0.93*	-	0.52*	1.8**	-	-	-	-	-	0.32*
	β_2		-2.9*	-4.6*	-	-	-2.4*	-8.7**	-	-	-	-	-	-
	r^2		0.60**		0.49*	-	0.61**	0.86**	-	-	-	-	-	0.43*
	21	50	15c	91a	69b	13c	79a	44b	37b	74a	75a	82b	93a	89ab
		78	23b	88a	80a	38b	86a	81a	48b	78a	77a	83b	92a	91ab
		101	60b	86a	87a	50b	90a	91a	62a	76a	86a	88a	91a	92a
		131	26b	91a	87a	47b	84a	88a	55a	75a	69a	83a	82a	87a
	β_1		3.0*	-		2.2**	0.87**	3.0**	1.4*	-	-	-	0.38*	-
	β_2		-15*	-	-	-9.8**	-4.4**	-14**	-6.6*	-	-	-	-2.8*	-
	r^2		0.47*	-		0.94**	0.65**	0.96**	0.71**	-	-	-	0.87**	-
	30	51	26b	88a	88a	45b	88a	92a	59b	78a	77a	89a	90a	93a
		79	81b	94a	92a	84c	95a	90b	72c	75ab	83a	83b	92a	90a
		98§	71b	89a	90a	85a	91a	89a	67a	76a	76a	82a	90a	88a
	β_1		8.7**	1.3*	-	5.1**	-	-	2.5*	5.0**	3.8**	-0.16*	-	-
	β_2		-52**	-8.9*	-	-29**	-	-	-16*	-34**	-26**	-	-	-
	r^2		1.0**		-	0.99**	-	-	0.76**	0.98**	0.73*	0.50*	-	-
	45	48	83a	83a	86a	84a	87a	82a	69a	51b	74a	85a	71b	84a
	γ_1¶		3.0**			1.4**	0.62**	1.8**	-	-	0.72**	-	-	0.36*
	γ_2		-150**	-	-25*	-75**	-31**	-82**	-	-	-40**	-	-4.4**	-16*
	γ_3		-	4.1*	4.2**	-	0.22**	1.43**	-	-	1.3**	0.60**	0.67**	0.91**
	γ_4		5.0**	-5.8**	-5.5**	3.1**	-	-	78**	180**	-1.9*	-0.84*	-2.3**	-1.4**
	γ_5		-	-95**	-	16**	-	-	11**	-43**	-	-	-	-5.6*
	r^2		0.83**	0.89**		0.93**	0.54**	0.81**	0.90**	0.44**	0.62**	0.43**	0.70**	0.35**

* and ** indicate significant at 0.01 and 0.05 levels, respectively.

† Values within a row for each storage temperature, seed moisture content, and seedlot followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's analysis and then back transformed for reporting.

‡ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted to each temperature, where y is the germination of switchgrass seeds without stratification (G), germination following stratification (SG), or germination following stratification and drying (SDG) and x is the seed moisture content (MC) under each temperature (T). When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters listed in table for β_2 has a factor of 10^{-3}. Parameters are listed as "-" when they are not significant at 0.05 level or not included in the regression models. β_0 is not listed.

§ Germination results is not presented for higher MC and T combinations which resulted in dying of most seeds after one year storage.

¶ Multiple regression equations ($y=\gamma_0+\gamma_1x+\gamma_2x^2+\gamma_3z+\gamma_4z^2+\gamma_5z*x$) were fitted across the temperatures, where y is G, SG, or SDG, x is MC, and z is T. Parameters for γ_2, γ_4,and γ_5 have a factor of 10^{-4}, 10^{-2}, and 10^{-3} respectively. γ_0 is not listed.

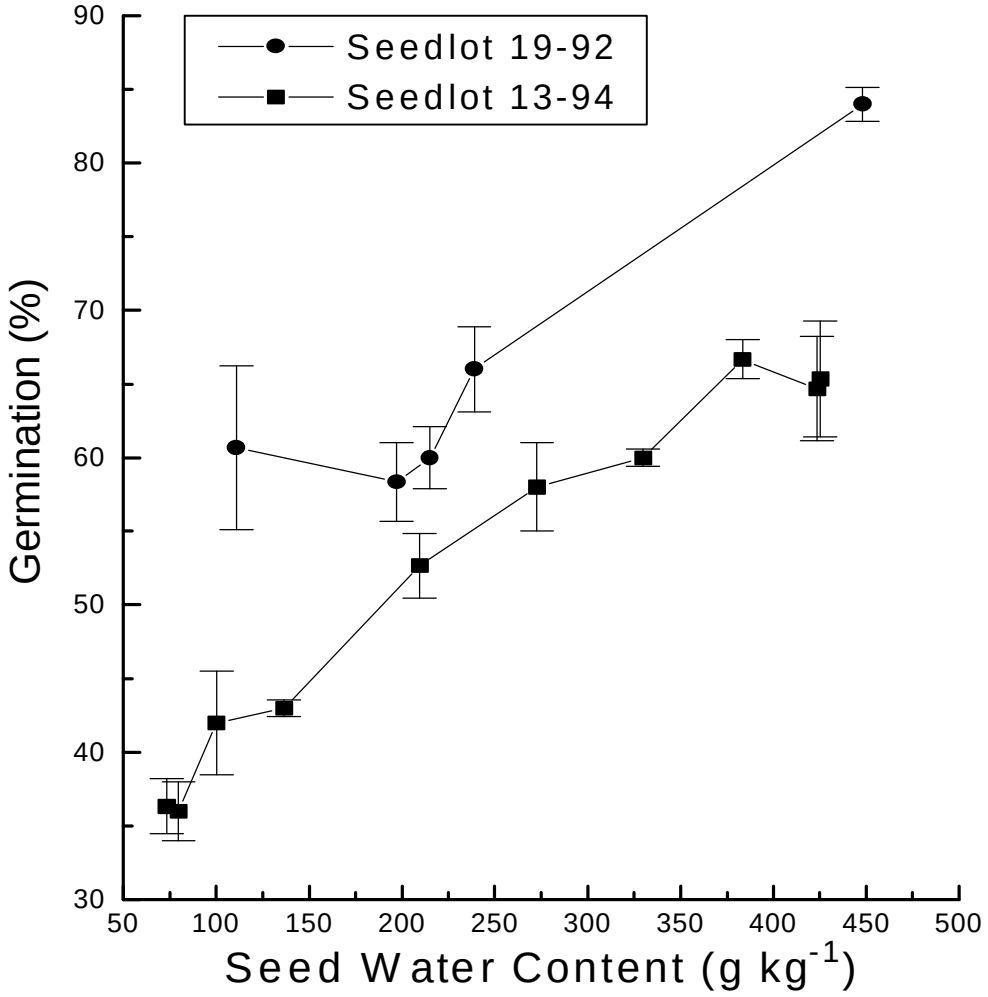

Fig. 4.1. The change in germinability of switchgrass seeds during drying following 2 weeks of stratification at 5 ^{o}C.

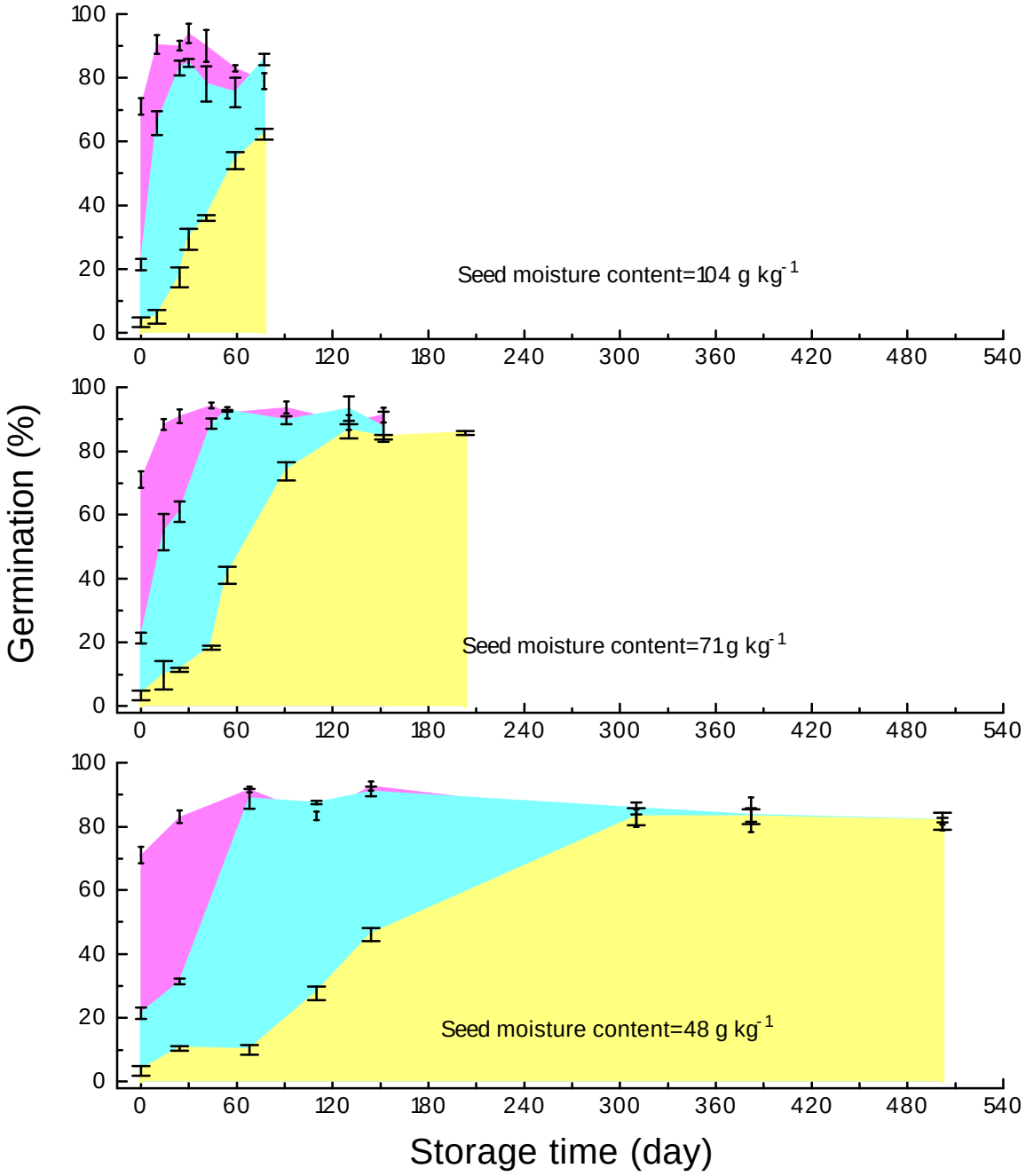

Fig. 4.2. The influence of seed moisture content and storage time on germination of switchgrass seedlot 13-94 stored at 45°C. The germination values include both normal and abnormal seedlings. Vertical bars indicate the standard errors. basal germination (G), germination after 2 wk stratification at 5°C (SG), germination after 2wk stratification at 5°C plus drying at room temperature in moving air for 3 d (SDG). SG and SDG were not observed on the last sampling date for 71g kg^{-1} due to the evident appearance of aging, which was reflected by the number of abnormal seedlings.

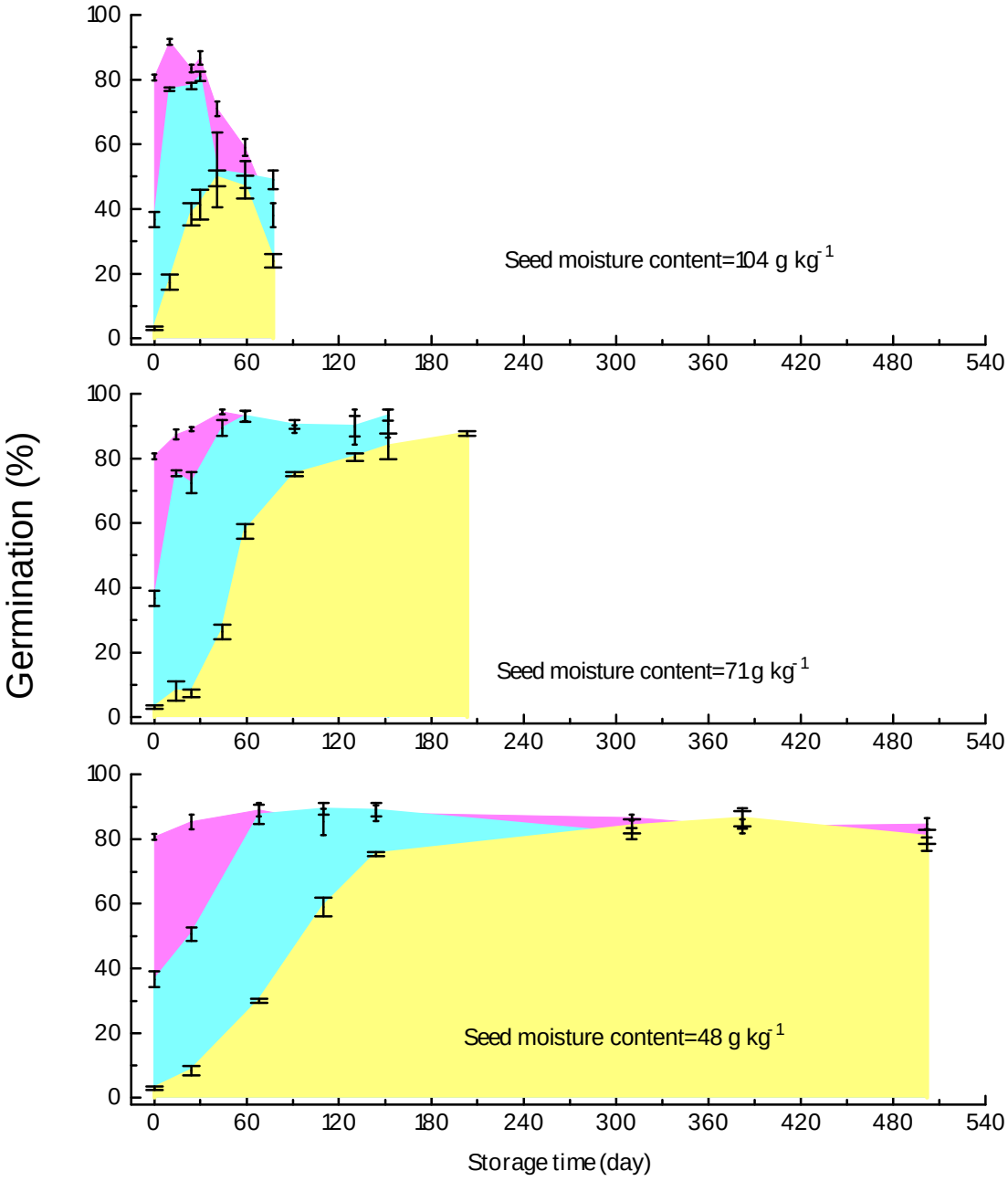

Fig. 4.3. The influence of seed moisture content and storage time on germinationof switchgrass seedlot 1-94 stored at 45°C. The germination values includeboth normal and abnormal seedlings. Vertical bars indicate the standard errors. basal germination (G), germination after 2 wk stratification at 5°C (SG), germination after 2wk stratification at 5°C plus drying at room temperature in moving air for 3 d (SDG). SG and SDG were not observed on the last sampling date for 71g kg^{-1} due to the evident appearance of aging, which was reflected by the number of abnormal seedlings.

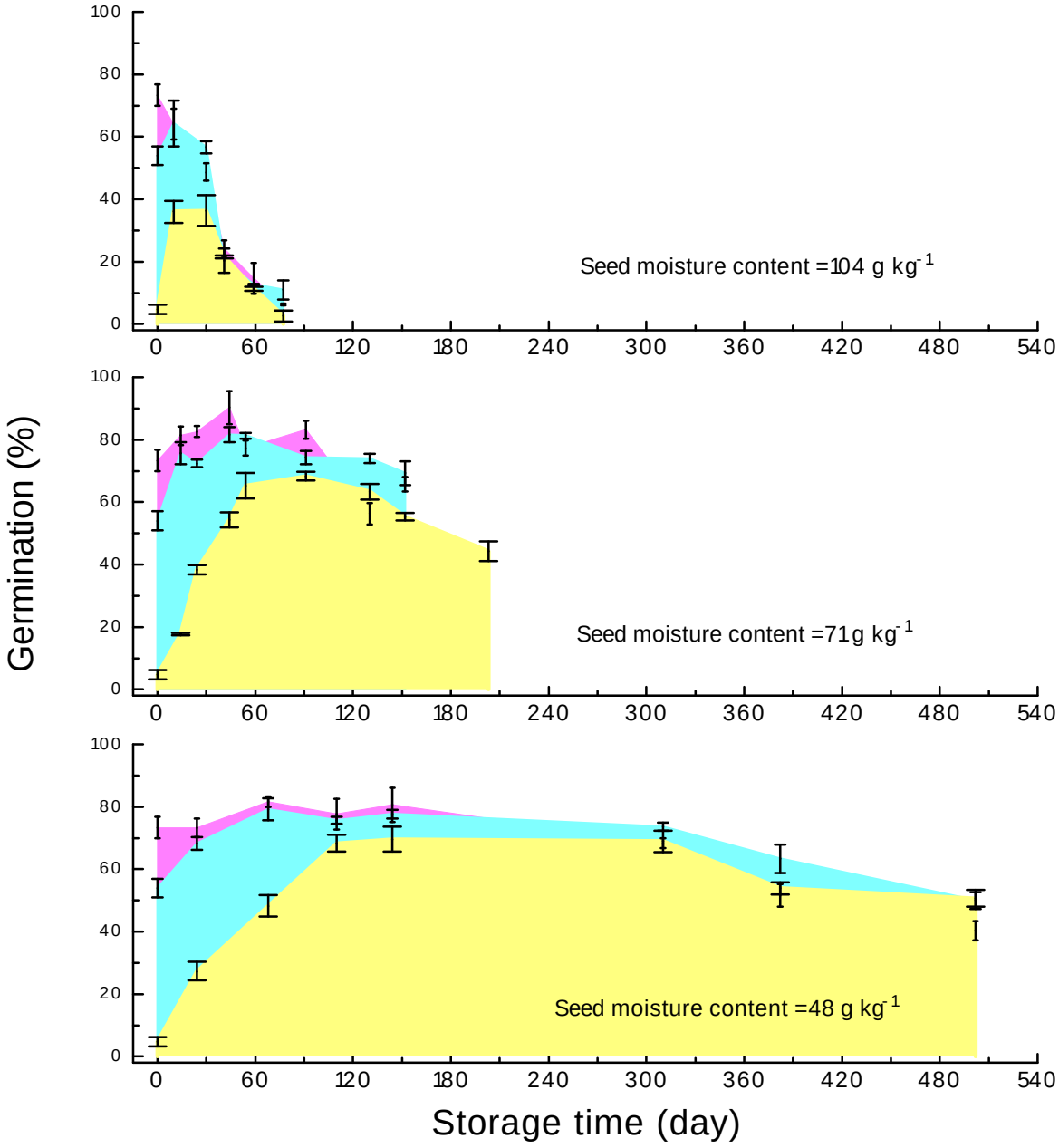

Fig. 4.4. The influence of seed moisture content and storage time on germination of switchgrass seedlot 12-93 stored at 45°C. The germination values include both normal and abnormal seedlings. Vertical bars indicate the standard errors. basal germination (G), germination after 2 wk stratification at 5°C (SG), germination after 2wk stratification at 5°C plus drying at room temperature in moving air for 3 d (SDG). SG and SDG were not observed on the last sampling date for 71g kg^{-1} due to the evident appearance of aging, which was reflected by the number of abnormal seedlings.

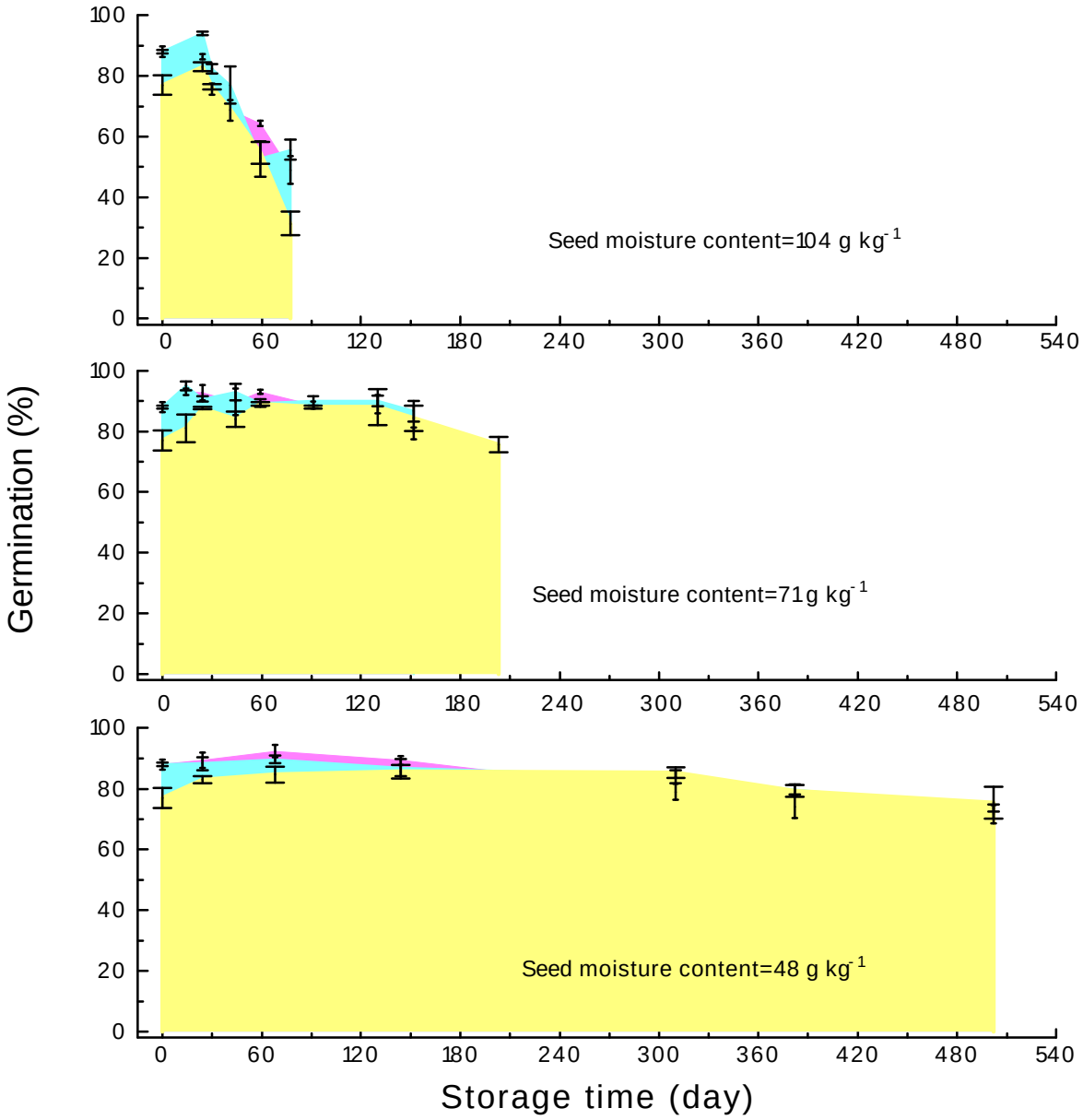

Fig. 4.5. The influence of seed moisture content and storage time on germination of switchgrass seedlot 1-92 stored at 45°C. The germination values include both normal and abnormal seedlings. Vertical bars indicate the standard errors. basal germination (G), germination after 2 wk stratification at 5°C (SG), germination after 2wk stratification at 5°C plus drying at room temperature in moving air for 3 d (SDG). SG and SDG were not observed on the last sampling date for 71g kg^{-1} due to the evident appearance of aging, which was reflected by the number of abnormal seedlings.

Chapter Five

The Influence of Temperature on Dormancy of Imbibed Switchgrass Seeds

ABSTRACT

This research was conducted to observe some temperature and time requirements for dormancy breaking and induction in imbibed "Cave-in-Rock" switchgrass (*Panicum virgatum* L.) seeds harvested in 1994 and 1995 from the Midwest and Virginia. Seeds of three seedlots were stratified at 5°C for 2 to 6 wk with or without pre-stratification conditioning at 21 or 30°C for 2 wk. Seeds of one seedlot were conditioned at 20°C for 2 to 17 wk and then stratified at 5°C for as long as 42 wk. Basal germination (G), germination following stratification (SG), and germination after post-stratification drying (SDG) were observed for seeds sampled at various times of conditioning. Many seeds conditioned at 21 or 30°C were induced into deeper dormancy, as evidenced by longer stratification requirements and greater post-stratification reversion (a reversible loss of germinability seen when stratified seeds are dried). Conditioning at the higher temperature caused a greater deepening of dormancy. Dormancy induction or deepening was not detected in some seedlots conditioned at 21°C for 2 wk if only G or SG was observed. Dormancy induction at 21°C was evident when SDG was examined. Newly harvested seeds with presumably greater innate dormancy were more readily induced into even deeper dormancy. At the beginning of prolonged wet storage at 20°C, some of the seeds within the seedlot might have actually been stratified and their dormancy broken but the majority were clearly driven deeper. Longer conditioning times induced deeper dormancy, which could be seen from longer stratification requirement and greater revertibility. When SG was observed for seeds conditioned more than 2 wk, dormancy induction at temperature around 20°C was evident. The dormancy of seeds increased with conditioning time at 20°C to such an extent that, after 17 wk of conditioning, SG was only 14% with 2 wk of stratification but went up to about 90% after being stratified for about 20 wk. Reversion increased with prolonged conditioning at 20°C and required even longer stratification periods for the deeper dormancy to be broken.

INTRODUCTION

Seeds of switchgrass seem to be unusually plastic in the range of treatments and factors that affect their germinability. They typically exhibit a high degree of innate dormancy when neoteric. This innate dormancy can be overcome to varying degrees by after-ripening (Chapters 2 and 3), stratification (Chapters 2, 3, and 4), and combinations of the two (Chapters 3 and 4). We have found that the dormancy-breaking effect of stratification can be at least partially reversed during post-stratification drying (Chapter 4). This reversion, as we call it, is a form of secondary dormancy, i.e., a dormant state that appears after mature seeds have demonstrated themselves to

be germinable.

A case has been built for the concepts of the plasticity of dormancy and germinability. It has also been suggested that the dormant and germinable states are not simply on and off, yes and no, conditions. Rather seeds of some species may be able to move back and forth over a range from fully or deeply dormant to fully or completely germinable. (Such species would most likely be ones where dormancy is modulated by internally variable physiological factors and not factors such as impermeable seedcoat or immature embryo). The deeper the dormancy, the greater or more extreme may need to be the measures to overcome it. The more germinable (less dormant) the seed, the less likely it is to slip back into a (more) dormant status, i.e., to develop secondary (or deeper) dormancy.

In this chapter, we will describe another apparent example of secondary dormancy or dormancy deepening in switchgrass--one that is induced by exposure of imbibed seeds to warm temperatures. The initial observations of this phenomenon came in studies on stratification where we were looking at seed responses to both time and temperature. We were surprised to find that "stratification" at temperatures above about 15°C broke very little dormancy or even seemed to cause seeds to become more deeply dormant, i.e., to require longer subsequent stratification periods.

Seed dormancy of many species besides switchgrass is highly plastic and can be induced, maintained, or released under various environmental conditions. Hydrated seeds, both dormant and nondormant, of some species can be induced into dormancy (or dormancy that requires extraordinary measures to relieve) by low oxygen or high carbon dioxide levels (Harper, 1957; Roberts, 1972). Dormancy can also be induced under aerobic conditions. Dormancy may be imposed by exposing some photoblastic seeds to white light, especially at high radiant flux densities, or to far-red light (Bartley and Frankland, 1982; Ellis et al., 1986). In many species, especially summer annuals, higher temperatures can induce dormancy, a sequence which is operationally opposite to the stratifying effect of lower temperatures (Baskin and Baskin, 1977, 1987; Bouwmeester and Karssen, 1993). A secondary dormancy induction in imbibed *Rumex crispus* seeds increases with prolonged exposure to temperatures between 1.5 and 15°C; and the efficacy of stratification decreases with time over this temperature range (Totterdell and Roberts, 1979, 1981). Although short periods of exposure to these temperatures cause a loss of innate dormancy, longer periods reverse the stratifying effects; induced secondary dormancy was evident in as little as 7 days at 15°C (Totterdell and Roberts, 1979, 1981).

Dormancy induction caused by extended incubation at lower temperatures has also been reported in other species in which dormancy can sometimes be released by stratification (Willemsen, 1975; Bouwmmeester and Karssen, 1993). Different responses to a stratification treatment can sometimes be observed within a single seed population due to genetic differences (Probert et al., 1989). Although dormancy-breaking effects of low-temperature incubation are common, especially among species adapted for spring germination, lower temperatures can induce dormancy in other species, particularly in species such as winter annuals which ideally germinate in autumn (Baskin and Baskin, 1986). In Japanese brome (*Bromus japonicus* Thunb.), secondary dormancy was induced by imbibition at 0°C (Haferkamp et al., 1994). Induced or secondary

dormancy, like innate dormancy, may in some cases be relieved by dry after-ripening and chemicals (Symons et al., 1986).

Switchgrass seeds commonly do not germinate at 30°C if not first stratified or after-ripened enough to overcome high levels of innate dormancy. We discovered rather serendipitously that most of those seeds that remain ungerminated at 30°C will not then germinate if stratified for 2 wk. Whether these seeds were dead or induced into deeper dormancy under warm and wet conditions was of great interest. It has also been observed that the efficacy of stratification decreases as temperature increases, and 22°C is the highest temperature at which stratification appears to occur (D.D. Wolf, unpublished results). The ordinal temperatures for secondary dormancy induction in switchgrass seeds are therefore of interest. The objectives of this research were to observe the temperature and time requirement for dormancy induction and to see if the newly discovered reversion phenomenon is helpful in investigating the contrasting effects of stratification and secondary dormancy induction.

MATERIALS AND METHODS

Seedlot Sources

Three seedlots of "Cave-in-Rock" switchgrass were used in these studies. Each exhibited a high degree of innate dormancy (usually less than 5% germinable). Seedlot 1-94 was hand-collected in September 1994 from the Whitethorne experiment station near Blacksburg, VA. Seedlots 15-94 and 18-95 were harvested by and purchased from a Midwest source (Allendan Seeds Company, Avoca, IA 51521) in the Falls of 1994 and 1995, respectively. An air separator was used to clean all seedlots so that average seed weight was greater than 1.85 mg/seed (540,000 seeds/kg). All seedlots were stored at 5°C after purchase (commercial seedlots) or harvest (hand-harvested seedlot).

The Influence of Stratifying or Conditioning Temperature on Dormancy

All three seedlots were used for dormancy-deepening studies. Seeds were "conditioned" for 2 wk at 21 or 30°C. Subsamples of 100 seeds were scattered on wet germination toweling, which was then rolled to form "ragdolls". The 23 by 31-cm towels weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in 17.7 by 20.3 cm plastic bags with zip closures. The plastic bags were then incubated for 2 wk at 21 or 30°C in the dark. One germination count was made and germinated seeds were discarded after 3 d at those temperatures. After the 2-wk conditioning period, ragdolls were moved to 5°C for 0, 2, 4, or 6 wk of stratification. Ragdolls with unconditioned seeds were also stratified at 5°C for 0, 2, 4, or 6 wk. At the end of the stratification period, two sets of ragdolls were removed, and one went directly to a 30°C-germinator for 10 d. Counts from this treatment represent germination following stratification (SG). The second set of ragdolls was dried at room temperature in moving air for 3 d. During that time, seed moisture content declined to about 100 g kg^{-1} (dry weight basis). Ragdolls were

then rewetted and germinated at 30°C for 10 d to observe germination following stratification and drying (SDG). Dormancy reversion is defined as the germination decrease caused by drying following stratification. In both cases (SG and SDG), one count was done after 3 d at 30°C to observe and remove the early germinated seed, and a final count was done after 10 d. Seedlings were considered normal germinants when both coleoptile and radicle were extended at least 5 mm. If the coleoptile or radicle protruded through the lemma and testa but failed to grow at least 5 mm, the seedlings were scored as abnormal germinants. Germination values presented in this chapter include both abnormal and normal seedlings.

The Influence of Stratifying or Conditioning Time on Dormancy

In a second study, ragdolls with 100 seeds each were made using seeds from seedlot 1-94. These were separated into 36 plastic bags with three ragdolls (replications) inside each. An incubator at 20°C was used for long-term conditioning. A germination count was made on all ragdolls, and germinated seeds were removed after 2 wk of incubation or conditioning at 20°C. Most ragdolls were returned for additional conditioning, but some were taken directly to a 30°C germinator or to stratification. After being conditioned at 20°C for 2, 6, 12, or 17 wk, various numbers of ragdolls were moved to 5°C to be stratified for up to 42 wk. SG and SDG were observed as above after each stratification time. Basal germination (G), which involved no stratification, was obtained by moving one plastic bag to 30°C for 10 d after conditioning at 20°C for 2, 6, 12, and 17 wk. No light (except occasional exposure during counting) was used during conditioning, stratification, or germination. (Preliminary studies showed that the germination of switchgrass was not influenced by light). As with the previous study, seedlings were considered germinants when either coleoptile or radicle was extended at least 5 mm. Therefore germination data presented in this chapter include both abnormal and normal seedlings.

Data Analysis

Analyses of variance for all germination variables were done with PROC GLM of SAS (SAS Institute, 1988). Germination data were converted to their square root and then arcsin transformed to comply with the basic assumptions of analysis of variance; back-transformed data (sin transformation followed by square) are used for reporting. Results are reported as significant when the P-value is 0.05 or less for Tukey's Studentized Range (HSD) test. The relationships between germination and stratification time were analyzed by regression methods.

RESULTS AND DISCUSSION

The Influence of Stratifying or Conditioning Temperature on Dormancy

Germination following conditioning and stratification (SG)

No differences for G (SG with 0 wk of stratification) were observed between 1-94, 15-94, and 18-95 whether unconditioned or conditioned at 21or 30°C (Table 5.1). Without stratification or further treatment, neither dormancy breaking nor induction could be seen at 21 or 30°C conditioning. A decrease of SG means fewer seeds could be made germinable with 2 wk of stratification. Longer stratification was required, i.e., the seeds were more deeply dormant. No SG difference was observed between unconditioned and 21°C-conditioned 1-94 seeds that were stratified for 2 wk. At 6 wk of stratification, SG was even higher for seeds conditioned at 21°C than for unconditioned seeds. Dormancy deepening was not readily seen for 1-94 at 21°C conditioning if only SG treatments were observed.

Dormancy deepening was evident with 30°C conditioning for all seedlots, i.e., dormancy was deepened and more seeds were in deeper dormancy, requiring longer periods of stratification for the dormancy to be broken. After 6 wk of stratification, seedlot 1-94 conditioned at 30°C remained less germinable compared with unconditioned or 21°C-conditioned seeds. After 2 wk of stratification, SG of 15-94 and 18-95 seeds conditioned at 21°C was significantly lower than unconditioned seeds. These two seedlots seemed to be more easily pushed into deeper dormancy by 21°C. After 4 wk of stratification, differences in SG between 21°C-conditioned and unconditioned seeds were significant only for 18-95. It seems that neoteric seedlots were more likely to have dormancy deepened by 21°C. Stratification of 15-94 and 18-95 for 6 wk broke the secondary dormancy induced by both 21 or 30°C. As the stratification time increased, the differences in SG of seeds caused by conditioning decreased. In other words, the deeper dormancy induced by higher conditioning temperatures was relieved by longer stratification.

Only seedlot 18-95 seemed to increase continuously in SG with stratification time. 18-95, as the most recently harvested seedlot, may have had more seeds that could benefit from extended stratification, i.e., were more deeply dormant. SG for 1-94 and 15-94 seeds that experienced conditioning at 30°C and stratification of 2 wk were 37% and 59%, respectively. After 2 wk of stratification, seedlot 18-95 conditioned at 30°C had only 27% seeds that would germinate, less than the lowest SG obtained for any neoteric seedlots without any conditioning treatment. Stratification for 2 wk was not effective in fully breaking the dormancy of seeds that had been conditioned at 21 and 30°C. In other words, both 21 and 30°C induced secondary dormancy or induced deeper dormancy. The germination increase by stratification for 2 wk was about two times higher for seeds conditioned at 21°C than at 30°C, i.e., 21°C was not as effective in inducing dormancy as 30°C.

Germination following conditioning stratification, drying, and rewetting (SDG)

Post-stratification drying reduced the germination of all seedlots with or without conditioning. Dormancy deepening was evident for all seedlots conditioned at both 21 and 30°C, since SDG was significantly lower for unconditioned seeds (Table 5.1). For seedlots 1-94 and 18-95 conditioned at 21 and 30°C, no difference was observed for SDG with 2 wk of stratification. A decrease of SDG means more seeds were reverting to dormancy during drying following stratification, i.e., more seeds were in a dormancy state where stratification was less quickly effective. Temperatures of 21 and 30°C were similarly effective in increasing dormancy for seedlots 1-94 and 18-95. After 4 wk of stratification, SDG of 1-94 and 18-95 conditioned at 21°C was higher than for seeds conditioned at 30°C, i.e., the dormancy deepening at 21°C was less intense than at 30°C. Stratification for 6 wk decreased the SDG differences between conditioning temperatures seen at 2 wk of stratification. Dormancy deepening by conditioning at 21 and 30°C was more evident by observing SDG then by observing SG, i.e., seeds were more easily pushed into some intermediate dormancy state than into deep dormancy.

SDG of all seedlots conditioned at 21 and 30°C increased between 2 and 6 wk of stratification (Table 5.1). For seeds that were not conditioned, only seedlot 15-94 showed a slight decrease of SDG. SDG of newly harvested seedlots 18-95 increased more than 50% between 2 and 6 wk of stratification. The innate dormancy of neoteric seeds required longer periods of stratification to break, and thus was deeper than seedlots that were more after-ripened.

The Influence of Stratification or Conditioning Time on Dormancy

Germination following stratification without drying (SG)

Four percent of the seeds in seedlot 1-94 germinated during 2 wk of conditioning at 20°C. When ragdolls were transferred from 20°C conditioning to 30°C germination for 10 d, G was 17%. That value was higher than the 11% germination of seeds that experienced neither conditioning nor stratification (significant at 0.10 level, statistics not shown). After 2 wk of stratification, SG for seeds conditioned at 20°C for 2 wk was 88%, which was higher than SG of 80% for seeds stratified for 2 wk but not experienced any conditioning (significant at 0.01 level, statistics not shown) (Table 5.1, Fig. 5.1). This suggests the 20°C conditioning may, under some circumstances, contribute to dormancy breaking. It is perhaps near some transitional point where stratification and dormancy deepening processes are both occurring. Longer times at 20°C did not show this duality.

After 6 wk of conditioning at 20°C, G was 7% when ragdolls were transferred directly (without stratification) to 30°C for 10 d. Stratification for 2 wk gave as SG of 69%. Germination went up to 78% with 4 wk of stratification and to a plateau of about 90% with 15 wk of stratification. That level was maintained with further stratification for up to 42 wk. When seeds were conditioned at 20°C for 6 wk, longer periods of stratification were required to bring the SG up to the level of those conditioned for only 2 wk.

After 12 wk of conditioning at 20°C, G was only 12% when ragdolls were transferred from 20°C to 30°C for 10 d. Stratification for 2 wk provided an SG of only 33%. Germination was 40%, 71%, 89%, and 93% with 4, 15, 20, and 36 wk of stratification, respectively. It is obvious that longer exposures to 20°C prior to stratification had the effect of deepening dormancy, requiring more stringent measures to restore germinability.

After 17 wk at 20°C, the germination was 14% when ragdolls were transferred from 20°C to 30°C for 10 d. Prolonged stratification resulted in germination similar to that after 12 wk of conditioning at 20°C. The levels of dormancy induced by 12 to 17 wk of conditioning at 20°C required stratification for 16 to 20 wk before the dormancy of all seeds was broken. (The plateau at about 90% for all treatment combinations likely was imposed by an assumed/supposed 10% of the seeds that were nonviable.)

Germination following stratification, drying, and rewetting (SDG)

Seedlot 1-94 had an SDG of 25% after being conditioned at 20°C for 2 wk and stratified at 5°C for 2 wk more (Fig. 5.1). After 4 wk of stratification, SDG was 41%. Stratification for 15 wk brought SDG up to 88%. Only at 15 wk did SG and SDG approach one another. The reversion process (during drying) was eventually no longer causing SDG to be lower than SG.

SDG was 13% after 6 wk of conditioning at 20°C followed by 2 wk of stratification. This further decrease in SDG as conditioning time increased was consistent with the decrease of SG. With another 2 wk of post-conditioning stratification, SDG was 21%. Stratification for 15 wk brought SDG to only 57%. Further changes in SDG were rather inconsistent and slow between 15 and 42 wk. The indication, again, is that exposure of dormant seeds to 20°C deepened their dormancy, requiring longer periods of stratification to overcome.

After 12 wk of pre-stratification conditioning at 20°C, when SG was 33%, SDG was only 6% with 2 wk of stratification. Stratification for 21 wk raised SDG to only 32%, but 36 wk of stratification raised SDG to almost 70%. This is remarkable for two reasons: the seeds must have been very deeply dormant to require such long periods of stratification, and seeds were still quite viable after almost 1 yr of hydration.

No difference between 12 and 17 wk of conditioning was observed for SDG when seeds were stratified for 2 wk; both were very low. With increasing stratification times, however, SDG of seeds conditioned for 17 wk was lower than seeds conditioned for 12 wk. At the longest stratification times tested for seeds conditioned for 6, 12, or 17 wk, the SDG was always lower than the corresponding SG. It was not clear if the differences between SG and SDG were due to deep dormancy or due to the possibility that some seeds were too weak to survive the post-stratification drying, or due to both. The fact that SDG was still rising at the last time tested suggested that the seeds were alive and their dormancy was continuing to be lessened by stratification.

SUMMARY AND CONCLUSIONS

Many seeds conditioned at 21 or 30°C were induced into deeper dormancy, as could be seen from longer stratification requirements and greater revertibility. The higher conditioning temperature conditioning was more effective in deepening dormancy. Dormancy induction or deepening may not be perceived in some seedlots if only G or SG is observed after conditioning. Dormancy deepening at 21°C was evident when SDG was examined. Newly harvested seeds with presumably greater innate dormancy were more readily induced into even deeper dormancy.

At the beginning of prolonged wet storage at 20°C, some of the seeds within the seedlot might have actually been stratified and their dormancy broken, but the majority were clearly driven deeper. Longer conditioning times induced deeper dormancy, which could be seen from longer stratification requirements and greater revertibility. When SG was observed for seeds conditioned more than 2 wk, dormancy induction at 20°C was evident. The dormancy of 1-94 seeds increased with conditioning time at 20°C to such an extent that, after 17 wk of conditioning, SG was only 14% with 2 wk of stratification but went up to about 90% after being stratified for about 20 wk. SDG decreased even more with prolonged conditioning at 20°C and required even longer stratification periods for the deeper dormancy to be broken.

All of these observations are consistent with a hypothesis that switchgrass seeds can be moved back and forth across a continuum of dormancy-germinability. Exposure to moderate temperatures can move seeds toward a more deeply dormant state as can post-stratification drying. Conversely, stratification and after-ripening can progressively (but reversibly) move seeds toward greater and greater germinability, where the possibility of reversion or high temperature-deepening of dormancy becomes less likely.

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Table 5.1. Germination of switchgrass seeds with or without post-stratification drying as influenced by pre-stratification conditioning temperature and duration of stratification.

Seedlot/ Estimates	Post-stratification drying	Stratification time	No conditioning	Conditioning temperature for 2 wk 21°C	30°C
		wk	----------%----------		
1-94	No (SG)	0	11a†	10a	8a
		2	80a	76a	37b
		4	91a	90a	64b
		6	85b	93a	71c
β_1‡			40**	37**	19**
β_2			-4.8**	-4.0**	-1.3*
r^2			0.98**	0.97**	0.96**
	Yes (SDG)	0	-	-	-
		2	56a	8b	6b
		4	57a	43b	19c
		6	74a	50b	-
β_1			-	11**	-
β_2			0.60**	-	-
r^2			0.66**	0.74**	-
15-94	No (SG)	0	18a	21a	26a
		2	93a	82b	59c
		4	91a	89ab	82b
		6	86a	92a	89a
β_1			40**	33**	20**
β_2			-5.0**	-3.6**	-1.6**
r^2			0.93**	0.95**	0.98**
	Yes (SDG)	0	-	-	-
		2	88a	24c	39b
		4	80a	49b	36b
		6	82a	83a	63b
β_1			-11*	15**	-23*
β_2			1.2*	-	3.6**
r^2			0.77**	0.95**	0.91**
18-95	No (SG)	0	4a	2a	4a
		2	82a	51b	27c
		4	86a	73b	53c
		6	87a	88a	90a
β_1			42**	26**	9**
β_2			4.9**	-2.1**	0.9*
r^2			0.94**	0.98**	0.99**
	Yes (SDG)	0	-	-	-
		2	34a	3b	3b
		4	47a	16b	7c
		6	76a	63ab	54b
β_1			-	-20*	-28**
β_2			1.3**	4.3**	5**
r^2			0.90**	0.97**	0.98**

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Tukey's multiple range test was used for comparisons of germination without conditioning, conditioning at 21°C, and conditioning at 30°C for each seedlot at each stratification time with or without post-stratification drying. Values within a row followed by the same letter are not significantly different at the 5% level. Original data were transformed for Tukey's test and then back transformed for reporting.

‡ Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted for each seedlot and conditioning treatment, where y was the germination of switchgrass seeds that had been stratified for 0 to 6 wk without or following drying, and x was the weeks of stratification at 5°C. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed.

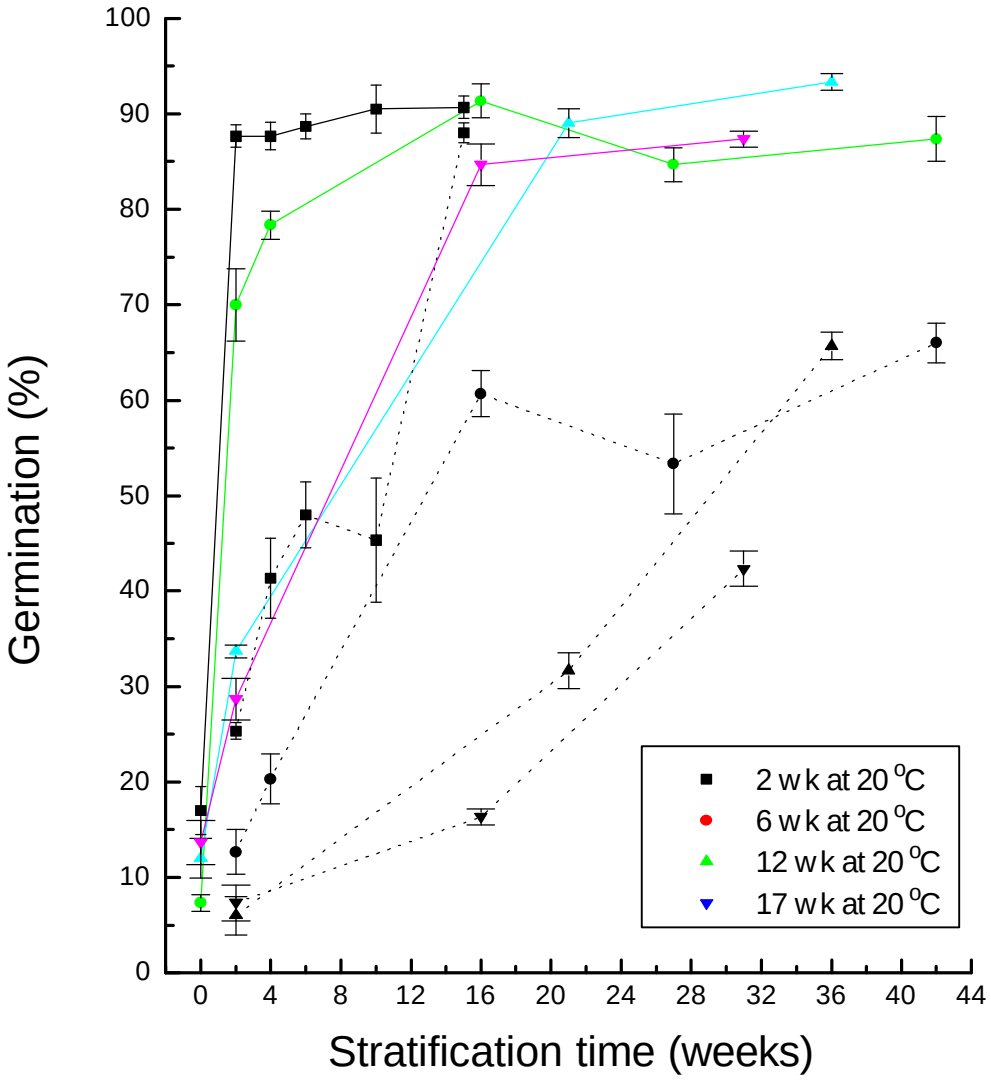

Fig 5.1 Germination of switchgrass seeds (seedlot 1-94) that had been conditioned at 20 °C for various durations, stratified at 5 °C, and then germinated without drying (solid lines), or with drying for 3 d and rewetting (broken lines). Vertical bars indicate the standard errors.

Chapter Six

Switchgrass Seed After-ripening and Aging as Influenced by Anoxia and Temperature During Storage

ABSTRACT

Reports vary on whether aging and after-ripening of seeds are O_2-requiring processes. One would like to minimize aging while after-ripening switchgrass (*Panicum virgatum* L.) seeds at elevated temperatures. This research was designed to see if aging could be reduced and after-ripening still accomplished by storing switchgrass seeds in N_2 at elevated temperatures. Highly germinable or neoteric highly dormant seeds were adjusted to 75 g kg^{-1} moisture content (MC) and stored at 60°C in jars vacuumed to 300 µm Hg, flushed with 99.99% pure N_2, sealed, and then enveloped in plastic bags also flushed with 99.99% pure N_2. Control treatments were set up by vacuuming, flushing, and enveloping the jar with air. In another experiment, seeds were adjusted to 55 g kg^{-1} MC and jars were vacuumed and flushed with N_2 or air before storing at 60°C without an envelope of plastic bags. Germinability of seeds with 75 g kg^{-1} initial seed moisture content (MC) increased from 4 to 65% in 13 d at 60°C in N_2. Germinability of the seeds in the air-flushed control increased to 58% at 7 d but decreased to 26% by 13 d. These results suggest less aging occurred in switchgrass seeds stored at 60°C in N_2, but after-ripening seemed to occur readily either in N_2 or air. Before aging became evident, no germination differences were observed between seeds stored in N_2 and air. After-ripening of switchgrass seemed not to be influenced by N_2 treatment. Germinability increased from 4% to about 80% in 32 d for seeds stored at 60°C and 55 g kg^{-1} initial MC whether in N_2 or air. After 32 d storage in N_2 or air, about one-half to one-third of seedlings were abnormal. After-ripening and aging of switchgrass seeds seem to be relatively insensitive to O_2 partial pressures at this lower MC.

INTRODUCTION

After-ripening is accelerated by O_2-enriched atmospheres and delayed in O_2-depleted ones in rice and wild oats (Roberts, 1962; Simmonds and Simpson, 1971). In rice, exposure to 100% O_2 approximately doubled the rate of after-ripening over that occurring in seeds gassed with N_2. Aerobic respiration, or at least oxidation, apparently is essential to the after-ripening process. The metabolism and growth associated with germination require an active synthesis of ATP. One hypothesis was proposed that dormancy was imposed by a block in energy metabolism and was broken by increased availability of phosphate acceptors such as glucose, presumably via the hydrolysis of stored forms (Ross, 1984). The conclusion that O_2 is required for dormancy breaking comes mostly from studies of the role of hulls and coats of imbibed seeds that are significant barriers to gas exchange (Black, 1959; Corbineau et al., 1986). However, none of the experiments were defined enough to prove that increased permeability to O_2 in turn enhanced availability of O_2 to embryo (Simpson, 1990).

Similar discrepancy exists for reports on aging difference between seeds stored in air and

anoxic conditions (Roberts, 1972a). Luo et al. (1983) reported peanuts (*Arachis hypogaea*) at 6.2% moisture stored in carbon dioxide (or N_2) at 38 to 40°C were afforded considerable protection in comparison with storage in air. Marzke et al. (1976), however, found negligible advantages for peanuts stored in these gases (6% hydration, 4 or 27°C) over those stored in air. Reviewers (Roberts, 1972b; Bass, 1973) have noted the considerable difficulties inherent in any assessment of the literature on the influence of various gaseous atmospheres on aging. Many reports of experimental protocols are inadequately detailed or indicate possible deficiencies in design. Poor control of seed moisture, in particular, has probably generated many false conclusions. As Ibrahim and Roberts (1983) have noted, a drop in MC of one percentage point can lead to a doubling of longevity in some cases.

The previous chapters reported after-ripening and aging of switchgrass seeds that were stored in paper bags (with controls for temperature but not seed moisture content) and sealed jars (with controlled temperature and seed moisture content). This chapter will discuss the possibility of breaking dormancy while minimizing aging by storing switchgrass seeds in anaerobic conditions.

MATERIALS AND METHODS

Seedlot Sources

Seeds of "Cave-in-Rock" switchgrass were used for this study. They typically exhibited a high degree of dormancy (usually less than 5% germinable) when newly harvested. Seedlot 13-94 was purchased from a Midwest source (Osenbaugh Grass Seeds, Lucas, IO 50151) in November 1993 and stored at 5°C until these experiments were performed in November 1995. Seedlot 1-92, which was high in germination, was composed of 1992-harvested seeds that had been naturally after-ripened for nearly two years during storage at room temperature. An air separator was used to clean both seedlots so that average seed weight was greater than 1.85 mg/seed (540,000 seeds/kg).

Storage

Seed moisture content (MC) of each seedlot was initially determined. Seed samples were weighed, dried in a 95°C oven for 36 hr, and reweighed. MC was determined on a dry weight basis, i.e., (Fresh Wt-Dry Wt)/Dry Wt. Two storage studies were performed. They used the same seedlots and 60°C storage but differed in seed MC.

Experiment One

Prior to placing seeds in high-temperature storage, their MC was adjusted to a common and uniform 75 g kg^{-1}. Based on the existing and target MC and seed mass, the amount of water to be removed was calculated. To draw away water, 80 g seeds of each seedlot were placed on a small electric balance inside a vacuum chamber. The changes of seed weight detected by the balance could be viewed through a window of the chamber. The pressure inside the vacuum

chamber was reduced to about 300 µm Hg. The ending point for vacuum drying was approximated by reading the balance inside the chamber and definitively established on a more sensitive balance outside the chamber. Each moisture-adjusted seedlot was immediately sealed in four layers of zip-closure plastic bags. Seed moisture was determined again before the flushing with N_2 .

Seeds of each seedlot were separated into two samples of about 40 g and placed inside small packets made by two layers of wipes. Two such packets, one for seedlot 1-92 and the other for seedlot 13-94, were placed in a Mason jar for either N_2 or air storage. Each jar was modified by installing two glass stopcocks. High vacuum grease was applied to stopcocks and the seal of the dome lid. The jars were connected to the chamber of a vacuum freeze drier via a rubber tube on the stopcock, and the pressure inside the jars was reduced to about 300 µm Hg. Jars were sealed and then pulled off the vacuum. Some jars were then connected through a flow meter to a gas tank with 99.99% pure N_2. N_2 was first released to flush air out of the tubing. The stopcock was then connected to the N_2 tube and opened gradually to allow N_2 to be drawn in without creating a sudden low pressure and drawing in air. After a positive pressure was established in the jar, the upper stopcock was opened, and N_2 was flushed in from the lower stopcock and out from the upper stopcock for 5 min at 3 L N_2 / min. The outlet stopcock was closed before the inlet stopcock so that there was a positive N_2 pressure inside the jar when N_2 flushing stopped. The vacuum and flushing process were repeated four times to minimize possible residual O_2.

To further minimize the possible leakage of air into the sealed jars during storage, four layers of plastic bags with zip-closures were used to enclose the jars. The inner plastic bag was flushed with N_2 for 10 min and left partially inflated, i.e., with a positive pressure of essentially pure N_2. N_2 was flushed and a positive pressure restored with N_2 whenever the plastic bags seemed to lose pressure (every 3 to 4 d). Other jars went through the vacuuming and flushing process exactly the same as the N_2-flushed jars, except that they were flushed and later contained in plastic bags with air that had been passed through a desiccant. Thus all jars were under a slight positive pressure but some were filled with air and others with essentially pure N_2. Jars were then placed at 60°C, and seed samples were withdrawn periodically for testing.

The germinability of N_2-or air-flushed seedlots stored at 60°C was characterized periodically using the "ragdoll" methods described below. MC was determined for each seedlot at each sampling time. MC was determined for two samples from each seedlot at each sampling time for germination tests. After sampling, the jars were vacuumed, flushed (with a final positive pressure), and resealed in plastic bags with N_2 or air as above.

Experiment Two

For this study, the target MC was 55 g kg^{-1}. The seedlots and procedures for MC adjustment, vacuuming, and flushing were the same as experiment one, except that the jars were not enveloped in N_2- or air- flushed bags.

The germinability of N_2-or air-flushed seedlots stored at 60°C was characterized periodically using the "ragdoll" methods described below. MC was determined for two samples from each seedlot at each sampling time for germination tests. The jars were vacuumed and flushed after each sampling time to restore the anoxic and "control" environments.

Post-Storage Treatments and Germination Tests

Upon removal from storage after various times, subsamples of 100 seeds were placed in rolled, wetted germination toweling ("ragdolls") and tested for their germinability either immediately or following various other treatments. The towels weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in 17.7 by 20.3 cm plastic bags with a zip closure. Ragdolls were placed in one plastic bag for subsequent treatments (either stratification or germination). No illumination was used during stratification or germination. (Preliminary studies showed that the germination percentage of switchgrass was not influenced by light).

Different treatment sequences were used before observing the germination response. Basal germination (G) was the value obtained by moving ragdolls directly from storage to germination at 30°C for 10 d. Germination following stratification (SG) was determined by first placing ragdolls at 5°C for 14 d and then transferring them to 30°C for 10 d. Germination following stratification and drying (SDG) involved stratifying seeds in ragdolls at 5°C for 14 d, drying in the ragdolls at room-temperature in moving air for 3 d, and then germination at 30°C for 10 d after rewetting. Seeds from the SDG treatment reached equilibrium with air and held less than 100 g kg^{-1} water after 3 d drying at room temperature in moving air. In all cases (G, SG, and SDG), one count of germination was done at 3 d to observe and remove the early-germinating seeds, and a final count was made after 10 d at 30°C. Seedlings were considered normal germinants when both coleoptile and radicle extended at least 5 mm. If the coleoptile or radicle protruded through the lemma and palea but failed to grow at least 5 mm, the seedlings were scored as abnormal germinants. Unless otherwise indicated, germination values reported are total germination, which includes both normal and abnormal seedlings. An "abnormal rate" of germinants was calculated as the percentage of total germinants that were abnormal.

Data Analysis

All germination tests were done in triplicate, i.e., three ragdolls per treatment. Analyses of variance for all germination variables were done with PROC GLM of SAS (SAS Institute, 1988). Germination data were converted to their square root and then arcsin transformed to comply with the basic assumptions of analysis of variance; back-transformed data (sin transformation followed by square) are used for reporting. Results are reported as significant when the P-value is 0.05 or less for Tukey's Studentized Range (HSD) test. The relationships between germination values and storage times were analyzed by regression method.

RESULTS AND DISCUSSION

Experiment One

The values of G, SG, and SDG were significantly higher for 1-92 and 13-94 seeds stored in N_2 than in air after 7 d at 60°C with MC around 70 g kg^{-1} (Table 6.1). After 13 d of storage, seeds stored in N_2 were again significantly more germinable than if stored in air, but the germinability of both storage treatments was falling. No significant differences in MC were

observed between air and N_2 storage except at 13 d for 1-92. The slightly higher MC at 13 d for N_2-storage could only support the conclusion that the higher germination was caused by N_2 and not MC, because a higher MC normally leads to faster aging and a decrease of germinability (Ibrahim and Roberts, 1983, also see Chapter 3). The germinability did decrease at the later sampling times for N_2 and air. N_2 flushing slowed the decrease in germination of seeds stored at 60°C. The higher percentage of abnormal seedlings in air storage for G of 1-92 at 13 d as well as for G, SG, and SDG of 13-94 at 7 d seems to suggest seeds aged faster in the presence of O_2.

Highly significant linear decreases of G were observed for seedlot 1-92 stored both in N_2 and in air (Table 6.1). The decrease of G was faster in air. The fitted line for N_2 treatment was shown be significantly different from that for air. The slower aging process for 1-92 flushed with N_2 was not caused by a decrease in MC. G of 13-94 increased in the first 7 d and decreased between 7 and 13 d in both N_2 and air. SG of 13-94 decreased both in N_2 and in air during the first 7 d storage, indicating likely aging. Less SG decrease was observed for seeds in N_2. SDG of 13-94 increased in the first 7 d of storage and seeds stored in N_2 went up more, probably because many more of the seeds stored in air had already died.

Experiment Two

Storage at 60°C and 55 g kg^{-1} MC caused seeds to after-ripen quickly and then to show evidence of aging. There was little effect, however, of anoxia. Whether in air or in N_2, seeds of 1-92 and 13-94 stored at 60°C and the lower MC did not differ significantly in G, SG, or SDG at almost all sampling times (Table 6.2). At 32 d, SDG and its abnormal seedling rate of 1-92 seeds stored with N_2 were higher than for seeds stored in air (Table 6.2). The decrease in G, SG, and SDG for 1-92 after 22 d as well as the heightened abnormal rate suggest that aging occurred even in N_2 at 60°C with MC around 55 g kg^{-1}. G of 13-94 kept increasing through 32 d, indicating after-ripening was occurring in air and N_2. The increasing abnormal rate, was clear evidence of aging also. SG and SDG of 13-94 decreased after 10 d of storage. The influence of aging by 32 d storage was so great that almost one-half and one-third of the seedlings were abnormal for SG and SDG, respectively.

Aging differences between air and N_2 were not seen as they were in the earlier study. These seeds were stored in jars that were not enveloped in N_2-flushed plastic bags. Some O_2 might have leaked into the N_2 flushed jar, but this seems unlikely since a positive N_2 pressure inside the jar was observed at almost all sampling times for germination tests (although the pressure was usually not as large as right after flushing). As a minimum, we can say that little air could have leaked into the jar. There had to be a many-fold difference in O_2 levels between the two treatments, and yet there was little or no difference between the two in the degree of after-ripening or aging.

By comparing the germination results from experiments one and two, it seems that after-ripening of switchgrass seeds was not influenced, while aging was slowed down when N_2 was flushed and care was taken to prevent air, thus O_2, from leaking into the system. It also seems that only a very small amount of O_2, if any, was needed to permit aging of switchgrass seeds. The possibility could not be excluded that the germination differences in experiment one and two were related to seed moisture differences. There are reports that after-ripening is accelerated by O_2-

enriched atmosphere and delayed in O_2-depleted ones in rice and wild oats (Roberts, 1962; Simmonds and Simpson, 1971). Considering the fact that a narrow MC range and only 60°C were tested in this research, the results must be considered somewhat preliminary and the work needs to be expanded.

SUMMARY AND CONCLUSIONS

Germinability increased from 4 to 65% in 13 d for 13-94 seeds stored at 60°C and 75 g kg^{-1} MC in N_2. Germinability of 13-94 seeds in air increased to 58% at 7 d and decreased to 26% by 13 d. Less aging was observed for switchgrass seeds stored in N_2. When aging was not evident, no germination differences were observed between seeds stored in N_2 and air. After-ripening of switchgrass at 60°C seemed not to be influenced by N_2 or air. Germinability increased to about 80% in 32 d for 13-94 seeds (with 55 g kg^{-1} starting MC) stored at 60°C in N_2 or air. After 32 d storage in N_2 or air, about one-half and one-third of seedlings for 1-92 and 13-94, respectively, were abnormal.

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Table 6.1. The influence of anoxic storge and storage time on the germination of two switchgrass seedlots. Seeds were stored at 60°C for varying times. The target moisture content for seeds was 75 g kg^{-1}.

Seed-lot	Anoxic storage	Days of storage 0	7	13	β_1†	β_2	r^2
		----------%----------					
1-92		Germination without stratification (G)					
	Yes	79a‡	68a	42a	-2.8**	-	0.86**
	No	79a	49b	10b	-5.2**	-	0.97**
		Abnormal rate for germination without stratification					
	Yes	5a	79a	89b	15.0**	-0.67**	0.99**
	No	5a	90a	100a	18.0**	-0.80**	0.99**
		Germination following stratification (SG)					
	Yes	94a	59a	-	-5.0**	-	
	No	94a	36b	-	-8.2**	-	
		Abnormal rate for germination following stratification					
	Yes	28a	85a	-	11.7**	-	
	No	28a	95a	-	12.8**	-	
		Germination following stratification, drying, and rewetting (SDG)					
	Yes	90a	49a	-	-6.0**	-	
	No	90a	34b	-	-8.1**	-	
		Abnormal rate for germination following stratification, drying, and rewetting					
	Yes	20a	72a	-	10.0**	-	
	No	20a	84a	-	11.6**	-	
		Seed moisture content (MC, g kg^{-1})					
	Yes	7.5a	7.4a	6.8a	-	-0.0041*	0.77*
	No	7.5a	6.7a	6.4b	-0.079*	-	0.73*
13-94		Germination without stratification (G)					
	Yes	4a	71a	65a	15.0**	-0.81**	0.99**
	No	4a	58b	26b	15.0**	-1.0**	0.99**
		Abnormal rate for germination without stratification					
	Yes	0a	57a	80a	10.0**	-0.32**	
	No	0a	76b	96a	15.0**	-0.59**	
		Germination following stratification (SG)					
	Yes	81a	73a	-	-1.1**	-	
	No	81a	61b	-	-2.8**	-	
		Abnormal rate for germination following stratification					
	Yes	0a	52b	-	7.4**	-	
	No	0a	75a	-	10.6**	-	
		Germination following stratification, drying, and rewetting (SDG)					
	Yes	37a	68a	-	4.4**	-	
	No	37a	53b	-	2.2**	-	
		Abnormal rate for germination following stratification, drying, and rewetting					
	Yes	0a	50b	-	7.1**	-	
	No	0a	74a	-	10.5**	-	
		Seed moisture content (MC, g kg^{-1})					
		7.6a	7.9a	6.7a	-	-	-
		7.6a	7.4a	6.3a	-	-0.008*	0.82*

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted to each seedlot stored with or without N_2 flushing, where y is germination, abnormal rate, or seed moisture content (MC) and x is the storage time. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models. β_0 is not listed.

‡ Value pairs within a column for each seedlot and time followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's analysis and then back transformed for reporting.

Table 6.2 The influence of anoxic storage on the germination percentage of two switchgrass seedlots. Seeds were stored for up to 32 d at 60 °C and a target moisture content of 55 g kg^{-1}.

Seed-lot	Anoxic storage	Days of storage 0	5	10	15	22	32	β_1†	β_2	r^2
1-92					Germination without stratification (G)					
	Yes	79a‡	87a	86a	86a	82a	69a	1.1**	-0.047**	0.68**
	No	79a	89a	88a	85a	87a	72a	1.2**	-0.047**	0.65**
					Abnormal rate for germination without stratification					
	Yes	5a	13a	10a	24a	34a	56a	-	0.048**	0.89**
	No	5a	9a	12a	21a	27b	44a	1.1**	-	0.94**
					Germination following stratification (SG)					
	Yes	94a	90a	84a	69a	-	60a	-1.1**	-	0.80**
	No	94a	89a	88a	67a	-	54a	-1.3**	-	0.89**
					Abnormal rate for germination following stratification					
	Yes	3a	10a	4a	12a	-	49a	-	0.043**	0.94**
	No	3a	10a	11a	18a	-	44a	1.3**	-	0.89**
					Germination following stratification, drying, and rewetting (SDG)					
	Yes	91a	90a	88a	-	-	70a	-	-0.020**	0.96**
	No	91a	93a	90a	-	-	61b	-	-0.030**	0.98**
					Abnormal rate for germination following stratification, drying, and rewetting					
	Yes	3a	5a	13a	-	-	63a	-	0.058**	0.99**
	No	3a	7a	10a	-	-	52b	-	0.046**	0.99**
					Seed moisture content (MC, g kg^{-1})					
	Yes	55a	58a	55a	48a	50a	49a	-0.026**	-	0.51**
	No	55a	56a	57a	47a	48a	48a	-0.030**	-	0.60**
13-94					Germination without stratification (G)					
	Yes	4a	10a	24a	49a	70a	83a	2.7**	-	0.95**
	No	4a	12a	31a	47a	75a	78b	4.0**	-0.044**	0.96**
					Abnormal rate for germination without stratification					
	Yes	0a	12a	11a	8a	17a	25a	-	-	-
	No	0a	13a	14a	14a	17a	11b	1.5*	-0.041*	0.27*
					Germination following stratification (SG)					
	Yes	81a	92a	91a	74a	-	78a	-	-	-
	No	81a	91a	93a	75a	-	77a	-	-	-
					Abnormal rate for germination following stratification					
	Yes	3a	10a	4a	12a	-	49a	-	0.016**	0.82**
	No	3a	10a	11a	18a	-	44a	-	0.020**	0.82**
					Germination following stratification, drying, and rewetting (SDG)					
	Yes	37a	79a	89a	-	-	78a	7.2**	-0.019**	0.96**
	No	37a	82a	89a	-	-	77a	7.7**	-0.20**	0.94**
					Abnormal rate for germination following stratification, drying, and rewetting					
	Yes	3a	5a	13a	-	-	33a	0.53**	0.015**	0.99**
	No	3a	7a	10a	-	-	29a	-	0.028**	0.99**
					Seed moisture content (MC, g kg^{-1})					
	Yes	52a	63a	60a	54a	54a	51a	-	-0.00073*	0.36*
	No	52a	58a	55b	50a	52a	49a	-	-0.00058*	0.43*

* and ** indicate significance at 0.01 and 0.05 levels, respectively.

† Quadratic equations ($y=\beta_0+\beta_1x+\beta_2x^2$) were fitted to each seedlot stored with or without nitrogen flushing, where y is the germination of switchgrass seeds without stratification (G), germination following stratification (SG), germination following stratification and drying (SDG), abnormal rate for G, SG, and SDG, or seed moisture content (MC) and x is the storage time. When the quadratic fit was not significant, a linear equation ($y=\beta_0+\beta_1x$) was fitted. Parameters are listed as "-" when they are not significant at 0.05 level, or not included in the regression models.

‡ Values within a column for each seedlot and storage time followed by the same letter are not significantly different at the 5% level based upon Tukey's multiple range test. Original data were transformed for Tukey's analysis and then back transformed for reporting.

Chapter Seven

Summary and Recommendations for Future Research

Establishment of switchgrass (*Panicum virgatum* L.) may fail due to seed dormancy. The change of seed dormancy over time in response to environmental conditions is of great interest to seed scientists as well as plant ecologists. With extensive observation of switchgrass seeds stored under a wide range of temperatures and seed moisture contents, the following conclusions were made:

1. After-ripening and aging of switchgrass seeds are influenced by seed moisture content and temperature. While aging increases continuously with increase in seed moisture and temperature, an optimum seed moisture content is observed for after-ripening. Evidence was also seen that the moisture optimum for after-ripening shifts downward as temperature increases. It is deduced that there could be an optimum temperature for after-ripening. In order to observe such an optimum temperature, relatively small temperature intervals over controlled seed moisture contents are suggested for further research.
2. Drying switchgrass seeds after-stratification may cause them to revert to dormancy if stratification time is not long enough. After-ripening reduces the dormancy and thus the minimum stratification period that is needed to prevent dormancy reversion. The physiological or biochemical changes happening during drying need to be investigated.
3. Dormancy is deepened in imbibed dormant switchgrass seeds held at temperatures favorable for germination (of nondormant seeds). As the temperature decreases, fewer seeds are induced into deeper dormancy while more seeds are stratified and become germinable. It would be very interesting to know if at certain temperatures, some seeds can be induced into deeper dormancy while others are stratified.
4. After-ripening of switchgrass seeds appears under some circumstances not to be O_2-sensitive. More temperatures and seed moisture levels need to be tested to see more fully the influence of O_2 on after-ripening as well as aging.

With proper attention to potential complications of the treatments, it is possible to break the dormancy of switchgrass seeds and greatly improve the chance of successful establishment. Stratification for 4 to 6 wk will produce highly germinable, non-reverting seeds when stratified seeds are dried. Likewise, after-ripening in controlled environments where both temperature and seed moisture can be maintained can produce good germinability and minimal vigor losses.

Appendixes

Appendix A. Endogenous Abscisic Acid (ABA) in Switchgrass Seed Embryos during After-ripening

MATERIALS AND METHODS

Seedlot Souces

Seedlot 1-94 of "Cave-in-Rock" switchgrass was used for this study. The seedlot had less than 5% germinable seeds when newly harvested in September 1994 from plots growing at Blacksburg, VA. Seeds of 1-94 were stored at 5°C until the initiation of a storage study in January of 1995. An air separator was used to clean the seedlot so that average seed weight was greater than 1.85 mg/seed (540,000 seeds/kg).

Storage

Seed moisture content (MC) was adjusted to 100 g kg^{-1} before seeds were placed in a sealed jar at 30 °C. (With slight change during storage, the actual average MC was 98 g kg^{-1}). MC was determined on dry weight basis, i.e., (Fresh Wt-Dry Wt)/Dry Wt. During storage for up to 14 mo, seeds were taken out of the jar and kept at 5 °C until being tested for the germination and ABA in March 1996. 0 time germination and ABA content were obtained from 1-94 seeds that had been stored at 5°C and 50 g kg^{-1} until March 1996. Seeds stored under such a cool and dry condition have very little of their dormancy broken and are thus essentially neoteric.

Germination Test

Subsamples of 100 seeds were placed in rolled, wetted germination toweling ("ragdolls") and tested for their germinability. The towels weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in 17.7 by 20.3 cm plastic bags with a zip closure. Plastic bags with ragdolls were placed at 30 °C for 10 d to observe germination percentage. Seedlings were considered normal germinants when both coleoptile and radicle extended at least 5 mm.

ABA Measurement

The content of ABA in switchgrass seed embryo stored at 30° C and 100 g kg^{-1} MC was measured. The lemmas and paleas of 150 seeds were removed by gentle grinding. The naked caryopsis were soaked in icy distilled water for 2 hr and then dissected into endosperm and embryo. Embryos were again stored in icy water during collection, blotted to dry, frozen in liquid nitrogen, and lyophilized. All lyophilized samples were extracted in 80% methanol containing 100 mg L^{-1} of butylated hydroxytoluene for 2 h at 4° C in the dark and centrifuged for 30 min at 10,000 X g. The supernatant was dried and stored at -80° C until further processing.

Indirect ELISA and calculation of ABA concentrations in samples were as described by Walker-Simmons (1987). Monoclonal antibodies (MAb) were purchased (Idetek, San Bruno, CA, USA). Standard solutions of (S)-(+)-ABA were prepared in 1 ml of methanol and diluted with TBS. A series of eight ABA standards over the concentration range of 50 to 400 pg/100 µl were assayed on each microtiter plate; the outer rows and columns were not used to improve uniformity. Three separate aliquots were measured for each sample.

Data Analysis

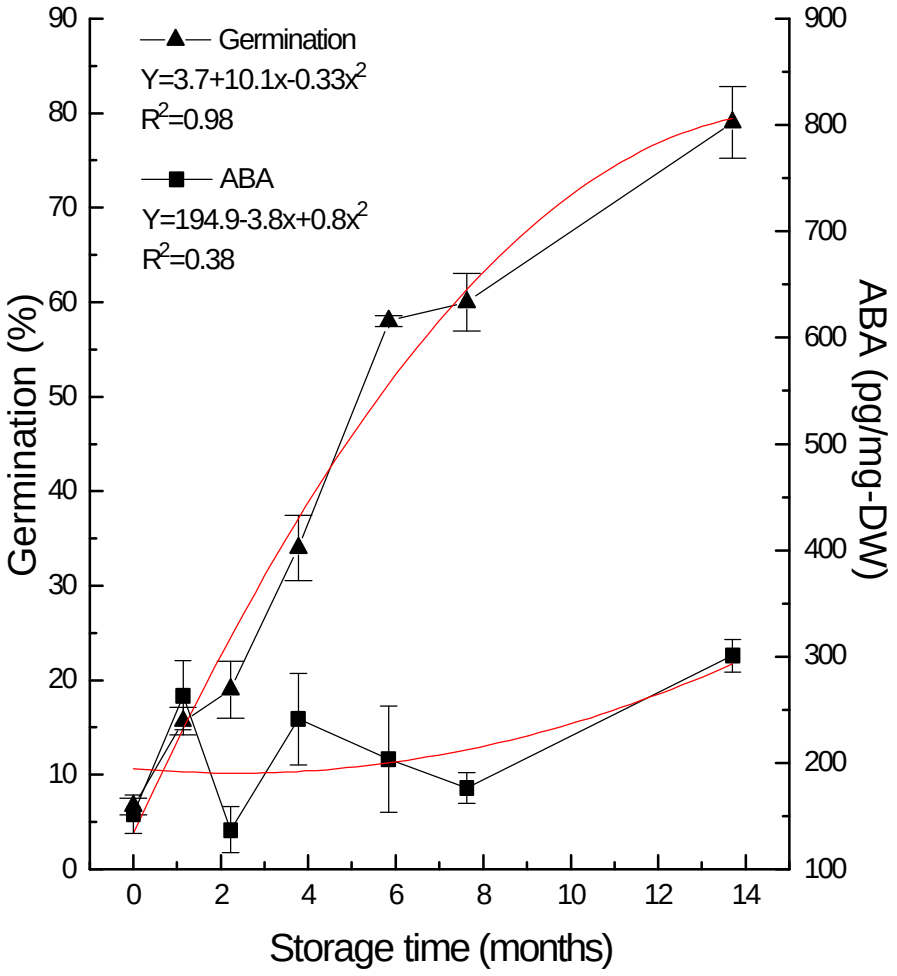
Polynomial regression were done to analyze the relationships between germination, ABA content, and storage times.

RESULTS AND DISCUSSION

Germination of switchgrass seeds increased from 3 to 80% while stored at 30° C and 100 g kg^{-1} MC for about 14 mo. The R^2 was highly significant for the germination-storage regression line. The change of ABA over storage time, was, however, nonsignificant (Appendix Fig.1.). The germination increase caused by after-ripening may not be simply related to the change of ABA, which is often believed to be able to inhibit germination and thus involved in dormancy of seeds.

REFERENCES

Walker-Simmons M. (1987). ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol. 84: 61-66



Appendix Fig.1. Germination percentage and embryo abscisic acid content for switchgrass seeds stored at 30 ^{o}C and 100 g kg^{-1} seed moisture content.

Appendix B. Responses to Gibberellic Acid (GA) and Abscisic acid (ABA) of After-ripenen Switchgrass Seeds

MATERIALS AND METHODS

Seeds of 12-93 seedlot were stored at 5 and 45 °C for 90 d before germination observations. Subsamples of 100 seeds were placed in rolled, wetted germination toweling ("ragdolls"). Ragdolls were wetted with 5or 50 µM GA or 10 or 40 µM ABA. Ragdolls wetted with distilled water were used as a check to calculate the germination response of seeds to ABA and GA. The towels weighed 4.5 g when dry and 13 g when wet. The ragdolls were placed in 17.7 by 20.3 cm plastic bags with a zip closure. Fifteen to 18 ragdolls were placed in one plastic bag for subsequent treatments (either stratification or germination). No illumination was used during stratification or germination. (Preliminary studies showed that the germination percentage of switchgrass was not influenced by light).

Different treatment sequences were used before observing germination responses. Basal germination (G) was the value obtained by moving ragdolls immediately to 30°C for 10 d. Germination following stratification (SG) was determined by placing ragdolls at 5°C for 14 d or more and then transferring them to 30°C for 10 d. Germination following stratification and drying (SDG) involved stratifying seeds in ragdolls at 5°C for 14 d or more, drying in the ragdolls at room-temperature in moving air for 3 d, and then germination at 30°C for 10 d after rewetting. In all cases (G, SG, and SDG), one count of germinants was done at 3 d to record and remove the early-germinating seeds, and a final count was made after 10 d at 30°C. Seedlings were considered normal germinants when both coleoptile and radicle extended at least 5 mm. The promotive response to GA was calculated as (germination in GA-germination in water)/germination in GA. The inhibitory response to ABA was calculated as (germination in water-germination in ABA)/germination in water.

Data Analysis

All germination tests were done in triplicate, i.e., three ragdolls per treatment. Mean separations were based on LSD at the 5% level of probability with PROC GLM of SAS (SAS Institute, 1988). Germination data were converted to their square root and then arcsin transformed to comply with the basic assumptions of analysis of variance; back-transformed data (sin transformation followed by square) are used for reporting.

RESULTS AND DISCUSSION

When germinated in distilled water, seeds stored at 45 °C for 90 days had SG similar to seeds stored at 5 °C, but G and SDG were higher. Storing seeds at 45 °C for 90 d released dormancy apparently without reducing the viability of seeds. A significant difference in SDG was seen between seeds stored at 5 and 45 °C when germinated with 5 µM GA. This may due to reduced variation for germination, because there was no germination increase compared to seeds germinated in distilled water. There was no difference for SDG and SG between seeds stored at 5

and 45 °C when germinated in 50µM GA. Germination was promoted for SDG by 50 µM GA. ABA was clearly inhibitory to germination at the higher concentrations tested.

REFERENCES

SAS Institute. 1988. SAS user's guide. Statistics. SAS Inst., Cary, NC.

Appendix Table 1. The response to gibberellic acid (GA) and abscisic acid (ABA) of switchgrass seeds following storage for 90 d at two temperatures.

Growth regulator			Germination			Germination change		
GA	ABA	Storage temperature	G	SG	SDG	G	SG	SDG
-------µM-------		°C	----------%----------					
0	0	5	4a†	60a	26b	-	-	-
		45	12b	64a	58a	-	-	-
$LSD_{0.05}$			8	11	6	-	-	-
5	0	5	4b	55b	25b	-19.1a	-5.5a	-6.0a
		45	18a	60a	64a	30.5a	-9.4a	10.2a
$LSD_{0.05}$			6	4	12	125.7	24.6	50.2
50	0	5	7b	65a	35a	34.4a	7.2a	26.1a
		45	22a	68a	57a	34.3a	5.9a	-6.1a
$LSD_{0.05}$			13	3.3	25	66.9	15.8	46.1
0	10	5	3a	46a	18a	3.9a	94.6a	30.9a
		45	8a	67b	48b	22.6a	88.1a	17.0a
$LSD_{0.05}$			8	11	18	245.6	12.7	47.2
0	40	5	0a	6a	3a	100.0a	100.0a	88.2a
		45	1a	12a	8b	90.4a	98.4a	85.6a
$LSD_{0.05}$			1.6	14.2	4.0	13.2	9.8	9.8

†Values followed by the same letter indicate no significant difference was observed between seeds stored at 5 and 45 °C. Mean separations were based on LSD at the 5% level of probability.

VITA

Zhengxing Shen, son of Hongyi Shen and Wenmei Tang, was born on 20 Dec. 1964 by lunar calendar, in Zhenjiang city, Jiangsu Province, mainland China. He received his first three years of education at his hometown in Haimen county, where he spent his happy early years. He finished his primary education in Shangdang Elementary School and part of his secondary education in Shangdang Middle School. He was graduated from Shanshan Middle School in July 1981. He was admitted to Nanjing Agricultural College (now Nanjing Agricultural University) in September 1981 and received a B.S. degree with a major in Agronomy in 1985. Later, he enrolled in the Graduate School of the same university and received an M.S. degree in Plant Breeding and Genetics in 1988. He worked for Anhui Academy of Agricultural Sciences between 1988 and 1993. In September 1993, he arrived at Blacksburg, VA to pursue a Ph.D. degree in Crop and Soil Environmental Sciences at Virginia Polytechnic Institute and State University. He is a member of the American Society of Agronomy and the Crop Science Society of America.

He has a younger brother, Chunxing Shen, who sometimes likes to claim to be silly.