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Response of Two Switchgrass (*Panicum virgatum* L.) Ecotypes to Seed Storage Environment, Storage Duration, and Prechilling[1]

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ABSTRACT

Inconsistent success in establishing switchgrass (*Panicum virgatum* L.) from seed is a major factor limiting its use. Freshly harvested seeds exhibit widely varying levels of innate seed dormancy. Studies were conducted to determine the response of 1995 and 1996 seed lots of 'Alamo' and a native collection (746) to storage environment, storage duration, and prechilling treatments. Initial mean germination of freshly harvested Alamo seeds averaged 24% and 76% for the 2 yr, whereas germination of 746 seeds were only 1% and <1%. The increased germination of the 1996 Alamo lot is mainly a factor of reduced innate dormancy levels; however, there was also an improvement in seed quality. Seeds of 746 responded positively to both a 14-d cold, moist prechill at 7 °C and higher storage temperatures, experienced in both the room temperature storage environment (approximately 21 °C) and during the spring and summer months in the warehouse environment (up to 38 °C). The response decreased with increased storage duration. Alamo showed little response to either storage environment or prechilling due to its reduced initial dormancy levels. Some dormancy was retained in 746 seeds throughout the 11-mo storage period in all environments; however, levels were higher in the seed cooler environment at 7 °C. Seed lots with an increased level of innate dormancy, such as 746, may require higher storage temperatures or prechilling before planting to ensure germination. Whereas, seed lots with a lower level of innate dormancy, such as the 1996 Alamo seed lot, will not benefit from such treatments.

INTRODUCTION

Switchgrass, a warm-season perennial grass native throughout much of the United States, is an important forage for both cattle and wildlife in the southern states (Grelen and Hughes, 1984), with significant potential for use as a bioenergy crop (Sanderson et al., 1996). Switchgrass exhibits a high degree of morphological variability, with two major recognized categories of ecotypes, upland and lowland, based largely on habitat preferences and some anatomical and genetic features (Eberhart and Newell, 1959; Hultquist et al., 1996; Sanderson et al., 1996). 'Alamo', a large, robust lowland cultivar, released by the USDA-Natural Resources

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Conservation Service (NRCS) James E. "Bud" Smith Plant Materials Center (PMC) at Knox City, Texas in 1978, has been tested at numerous sites in the south central and southeastern USA.

These evaluations have shown Alamo to be the most widely adapted commercial cultivar throughout this region (Sanderson et al., 1996). Under favorable conditions, Alamo grows too tallfor some potential uses, which has led personnel at the USDA-NRCS Jamie L. Whitten PMC at Coffeeville, Mississippi to examine numerous switchgrass accessions collected from Mississippi and Arkansas to search for shorter growing, upland ecotypes more suitable for forage production. Preliminary observations indicate that seeds of many of these ecotypes do not germinate as readily as Alamo.

Freshly harvested seeds of switchgrass have varying levels of dormancy that can adversely affect establishment (Jensen and Boe, 1991; Panciera et al., 1987; Sanderson et al., 1996; Zarnstorff et al., 1994). This dormancy has been attributed to physical restriction by the lemma and palea, because seeds can be induced to germinate by scarification that removes or weakens these structures (Haynes et al., 1997; Jensen and Boe, 1991; Sautter, 1962; Shaidaee et al., 1969); however, there is also evidence that germination inhibitors in the embryo are involved in the maintenance of dormancy (Zhang and Maun, 1989). High storage temperatures, prolonged storage, and moist prechilling have all been shown to reduce dormancy and improve germination of switchgrass seeds (Aho et al., 1989; Zarnstorff et al., 1994; Zhang and Maun, 1989).

Because of the growing interest in using switchgrass for forage, erosion control, and other conservation uses, there is a need for increased understanding of the seed germination characteristics of this grass. Objectives of this study were to determine: 1) the relative levels of seed dormancy of Alamo and 746, a Mississippi-collected accession of switchgrass; and 2) the effect of storage environment, storage duration, and prechilling on germination of these two switchgrass ecotypes.

MATERIALS AND METHODS

Seeds were collected (multiple harvests) in late Sept to early Oct. 1995 and 1996 from two germplasm sources, NRCS accession 9062746 (746) and Alamo, growing at the Jamie L. Whitten PMC. Accession 746 was collected as crown pieces in Grenada County, Mississippi in 1992, and the plants appear to conform to the morphological characteristics of an upland ecotype (Eberhart and Newell, 1959; Sanderson et al., 1996); however, no genetic testing was done to verify this (Hultquist et al., 1996; Sanderson et al., 1996). In 1996, 746 plants were previously cut for a forage harvest and seeds were produced on regrowth from the plant crowns. Florets (seeds) were released from the spikelets by use of a brush machine (Westrup a/s, Slagelse, Denmark), and a South Dakota Seed Blower (Seedburo Equipment Co., Chicago, IL) was used to remove chaff and under-developed seeds. After cleaning (<30 days after harvest), initial germination percentages for each seed lot were determined using five replications of 50 seeds that were given no pre-treatment prior to testing. Each lot was divided into thirds and placed in manila seed envelopes for storage. Envelopes of each ecotype were placed in the following storage environments: 1) Cooler (C) – a seed cooler with a controlled atmosphere of 7 °C with 55 to 60% relative humidity; 2) Room temperature (R) – a laboratory maintained at approximately 21 °C with ambient humidity levels; and 3) Warehouse (W) – an unheated storage facility with temperatures ranging from approximately -1 to 38 °C with ambient humidity levels. Pre-germination seed treatments consisted of prechilling (PC) at 7 °C for 14 d prior to germination testing and no prechilling (NC).

Germination tests were conducted every 2 mo from Dec. to Oct., with the target date being the first day of the month; however, actual planting dates often varied by several days from the target date due to scheduling realities. Tests extended over two calendar years, but for simplicity study years are referred to as 1996 and 1997. Due to multiple seed harvests and the length of time required for prechilling, storage durations cited are generalizations and do not refer to specific time periods. For example, seeds for the Dec. tests were placed in the cooler for prechilling during Nov., and storage duration for this test date is therefore referred to as 1 mo, although seeds were actually harvested 1 1/2 to 2 mo prior to the germination test. Treatments were arranged as a split-split-split plot, with storage environment as a whole plot, ecotype as a subplot, storage duration as a sub-subplot, and pre-germination seed treatment as a sub-sub-subplot. Five replications were used, with each consisting of 50 seeds from a single storage/pre-treatment combination, placed between two layers of moistened filter paper in a petri dish. The petri dishes were placed in a germinator (Hoffman Manufacturing Co., Albany, OR) for 14 d with alternating day/night temperature (30/15 °C) and (8 h/16 h) (AOSA,1993). Filter paper was moistened as needed with distilled water to ensure adequate moisture for germination. Germinated seeds were counted and discarded at 7 and 14 d (AOSA, 1993). Bewley and Black (1994) defined germination as emergence of the radicle, signaling the end of the germination process and the initiation of growth. Similar criteria were used in this study, where an individual seed was considered germinated when the radicle had protruded through the seed coat. No attempt was made to determine whether non-germinated seeds were viable. Data were subjected to an analysis of variance using the Statistical Analysis System (SAS) statistical package (SAS Institute, 1996). Means were separated using Fisher's protected least significant difference (LSD) ($P=0.05$). Because none of the error terms in the model differed significantly ($P<0.05$), they were pooled for analyses.

RESULTS AND DISCUSSION

Due to significant interactions between study years and other factors, data from each year were analyzed separately. Data presented are the total mean germination percentages after 14 d in the germinator. In 1996, an ice storm during the February testing period (3 mo) caused a power outage resulting in excessive drying of filter paper in the petri dishes; consequently this date was excluded from analysis.

Initial germination percentages for 746 were 1% and <1% for the 1995 and 1996 seed lots, respectively. The forage harvest during 1996 did not appear to affect dormancy levels in this ecotype. Aho et al. (1989) found that dormancy of 'Cave-in-Rock' seeds was not affected by cutting the plants for an early-season hay crop. Initial germination percentages for Alamo were 24% and 76% for 1995 and 1996 lots, respectively; however, in 1996, seeds in one of the petri dishes appeared to be adversely affected by an unidentified pathogen; if this replication were disregarded, initial germination increased to 83%. Sanderson et al. (1996) reported that recurrent selection for reduced dormancy within Alamo has led to germination rates for freshly harvested seeds approaching 50%. The initial germination percentage of 76% for the 1996 seed lot greatly exceeded this value. Location of seed production has been shown to affect dormancy levels of seeds from individual switchgrass cultivars (Panciera, 1983; Panciera et al., 1987). Alamo seeds produced in a warm, temperate climate, such as Mississippi, would likely possess reduced dormancy compared to seeds produced in cooler climates. Also, the seed cleaning process that removed lighter weight seeds has been shown to have a beneficial effect on germination percentage (Haynes et al., 1997).

Seed quality of both ecotypes harvested in 1995 was affected by ergot (*Claviceps* sp.) infection, and uneven ripening, which increased the number of immature, non-viable seeds. The South Dakota seed blower was not capable of removing seeds containing ergot and did not remove all immature seeds. After cleaning, the 746 seed lot contained >37% ergot-infected seeds. Alamo seeds were less affected by the disease; the seed lot contained only about 3% seeds with visible ergot. The number of non-viable seeds could not easily be determined, but appeared to be comparable between the two ecotypes. Both problems may be a function of high temperatures and low rainfall during the critical seed-ripening period in September 1995 (Table 1). Seeds that were obviously infected with ergot or of poor quality were replaced in the petri dishes before testing.

Table 1. Average monthly temperatures and rainfall recorded at Coffeeville, MS during the seed production and storage periods.

Yr		Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
1995	High Temp, °C	-----	-----	31	31	32	34	36	37	36	25	16	12
	Low Temp, °C	-----	-----	-3	0	8	11	18	21	7	8	1	-1
	Rainfall, mm	-----	-----	175	180	70	111	141	113	17	86	101	179
1996	High Temp, °C	11	13	16	23	29	30	32	31	28	24	16	15
	Low Temp, °C	-2	1	2	7	17	19	21	19	15	9	4	2
	Rainfall, mm	144	73	132	151	99	229	122	89	191	166	168	199
1997	High Temp, °C	10	14	21	21	26	29	33	31	29	---†	-----	-----
	Low Temp, °C	-2	3	6	7	12	18	21	19	16	-----	-----	-----
	Rainfall, mm	80	182	313	104	80	142	142	146	36	-----	-----	-----

† No data presented because final test was placed in the germinator on 30 Sept. 1997.

Germination percentages of 746 were lower than Alamo for most storage periods in 1996 (Table 2), indicating that it has a higher level of innate dormancy. The high germination percentage of Alamo at the shortest storage duration is noteworthy. Germination of freshly harvested seeds was only 24%; however, when averaged over all storage conditions, germination of 1-mo NC Alamo seeds was 59%, more than a two-fold increase. Germination of similarly aged NC 746 seeds averaged only 6.

Table 2. Influence of storage environment and duration on mean 14-d germination of two switchgrass ecotypes exposed to two pre-germination seed treatments with testing beginning in December of 1995 and ending in October of 1996.

			Storage duration (mo)†				
Ecotype	Storage Environment‡	Treatment§	1	5	7	9	11
			-----	-----	%	-----	-----
746¶	Room	NC	8	51	54	53	64
		PC	57	81	75	52	79
Alamo		NC	73	89	86	84	84
		PC	78	83	86	77	84
746	Cooler	NC	6	3	6	8	9
		PC	47	54	77	11	66
Alamo		NC	60	69	81	62	83
		PC	37	73	82	76	75
746	Warehouse	NC	4	13	35	49	63
		PC	48	57	80	54	79
Alamo		NC	45	78	71	75	73
		PC	68	68	79	75	84

Ecotype x storage environment x storage duration x pre-germination seed treatment LSD (0.05) = 14

† 3 mo storage duration not reported because of germinator failure.

‡ Cooler, seed cooler with controlled atmosphere of 7 °C, dehumidified to 55-60% RH; Room, laboratory with temperatures approximately 21 °C and ambient humidity levels; Warehouse, unheated warehouse with temperatures ranging from approximately -1 to 38 °C and ambient humidity levels.

§ PC, moist prechill at 7 °C for 14 d prior to placement in the germinator; NC, no prechilling prior to placement in the germinator.

¶ 746, NRCS accession 9062746 collected in Grenada County, MS.

There was a significant ecotype X storage environment X storage duration X pre-germination seed treatment interaction in 1996 (Table 2). Germination of both ecotypes was higher for seeds stored in the R environment and for later storage periods in W (summer) than for seeds stored in C. Dormancy of several native grass species has been shown to be retained longer in low temperature, low humidity storage (Coukos, 1944), such as in the C and the first 4 or 5 mo in the W environment and it has been widely reported that dormancy in switchgrass seeds is overcome more quickly at higher storage temperatures (Aho et al., 1989; Jensen and Boe, 1991; Zarnstorff et al., 1994). The lack of response to storage temperatures exhibited by Alamo is a function of the reduced dormancy of this ecotype, which is similar to the response Zarnstorff et al. (1994) found for 'Blackwell' seed lots with low levels of innate dormancy. The increased germination of the NC 746 seeds after 5 mo in the R environment indicated that this combination of storage time and conditions was sufficient to overcome much of the innate dormancy of this seed lot (Table 2).

Accession 746 responded positively to PC, whereas improvement in Alamo germination was none to slight from this treatment (Table 2). Other researchers have shown that switchgrass seeds with high levels of innate dormancy respond favorably to PC (Panciera et al., 1987; Zarnstorff et al., 1994). The response of 746 to PC decreased with increasing storage duration in the R and W storage environments. This pattern has been exhibited by seeds of other switchgrass cultivars (Aho et al., 1989; Haynes et al., 1997; Jensen and Boe, 1991; Zarnstorff et al., 1994), and indiangrass [*Sorghastrum nutans* (L.) Nash] (Emal and Conard, 1973). According to Zhang and Maun (1989), germination inhibitors in the embryo decay during storage, releasing seeds from dormancy; however, the rate of decay is temperature dependent. In this study, the increased temperatures in the R and during the summer in the W storage environments would result in an increased rate of decay. Seeds of 746 stored for 11 mo in all environments responded significantly to PC, indicating that dormancy was still present, however, the level was higher for seeds stored in the C environment.

In 1997, there were significant ecotype X storage environment X storage duration (Fig. 1), ecotype X storage environment X pre-germination seed treatment (Fig. 2), and ecotype X storage duration X pre-germination seed treatment interactions (Fig. 3). The underlying causes of these interactions are similar to those discussed for the four-way interaction in 1996. Alamo again exhibited higher germination percentages than 746; however, seed quality was better in this year. There was <1% obviously ergot-infected seeds for both ecotypes, fewer immature seeds, and germination of both ecotypes at most testing dates was higher than in 1996.

Germination of 746 was higher after storage in R and W (later storage periods), but the magnitude decreased with increasing storage duration (Fig. 1). The fairly high germination percentage of 3-mo 746 seeds in the R environment appears to indicate that this storage period, which was excluded from the 1996 analysis, was sufficient to overcome a significant amount of seed dormancy. Alamo exhibited little response to storage environment or duration due to its reduced innate dormancy levels. The PC treatment increased germination of 746 seeds, but the effect was not as pronounced in the R environment (Fig. 2) or at later storage durations (Fig. 3). The PC treatment did not appear to be beneficial for Alamo seeds, again due to their lack of dormancy.

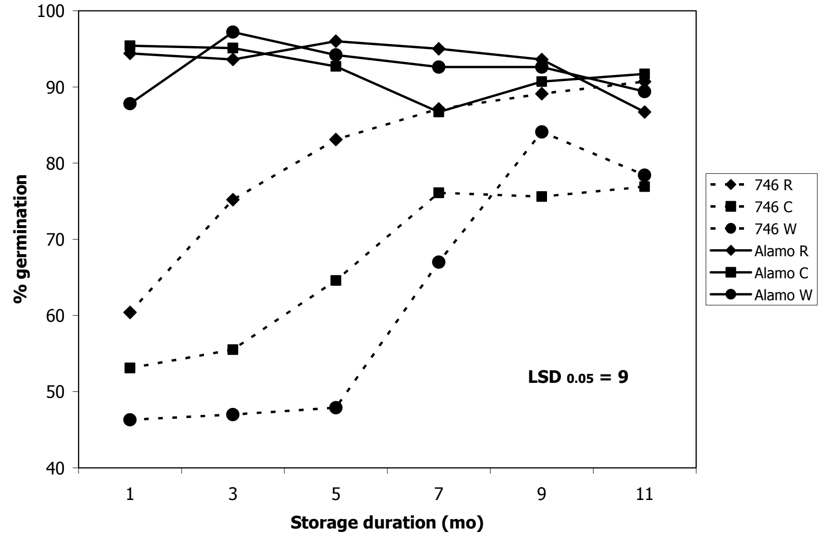

Fig. 1. Mean germination percentages of two switchgrass ecotypes after 14 d in the germinator as affected by storage environment and duration with germination testing beginning in December 1996 and ending in October 1997. Storage environments used were C, seed cooler with controlled atmosphere of 7 °C, dehumidified to 55-60% RH; R, laboratory with temperatures approximately 21 °C and ambient humidity levels; W, unheated warehouse with temperatures ranging from approximately -1 to 38 °C and ambient humidity levels. Ecotype 746 is NRCS accession 9062746 collected in Grenada County, MS.

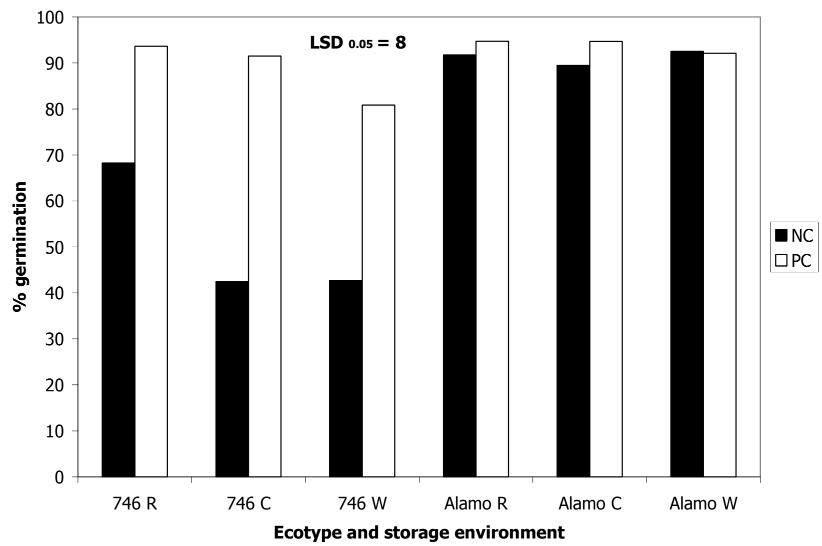

Fig. 2. Mean germination percentages of two switchgrass ecotypes after 14 d in the germinator as affected by storage environment and pre-germination seed treatment with germination testing beginning in December 1996 and ending in October 1997. Storage environments used were C, seed cooler with controlled atmosphere of 7 °C, dehumidified to 55-60% RH; R, laboratory with temperatures approximately 21 °C and ambient humidity levels; W, unheated warehouse with temperatures ranging from approximately -1 to 38 °C and ambient humidity levels. Ecotype 746 is NRCS accession 9062746 collected in Grenada County, MS. Pre-germination seed treatments used were prechilling (PC) seeds at 7 °C for 14 d prior to placement in the germinator and no prechilling (NC).

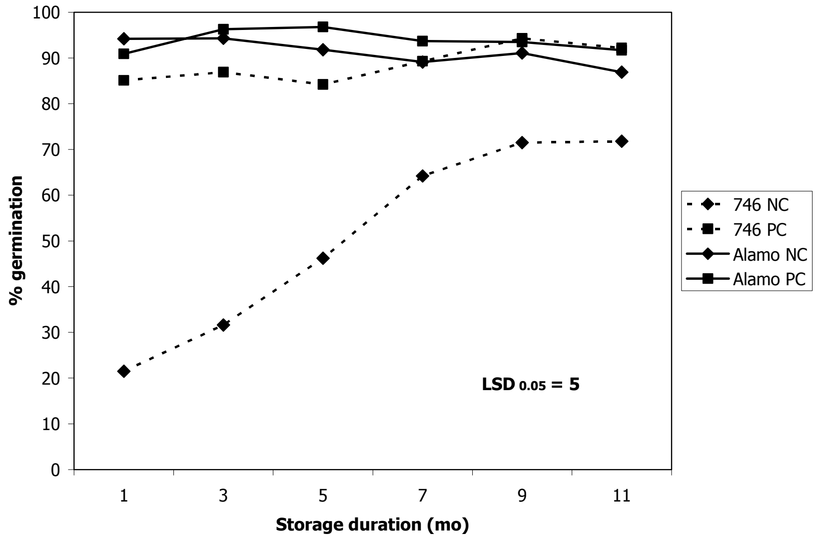

Fig. 3. Mean germination percentages of two switchgrass ecotypes after 14 d in the germinator as affected by storage duration and pre-germination seed treatment with germination testing beginning in December 1996 and ending in October 1997. Ecotype 746 is NRCS accession 9062746 collected in Grenada County, MS. Pre-germination seed treatments used were prechilling (PC) seeds at 7 °C for 14 d prior to placement in the germinator and no prechilling (NC).

It appears that much of the inconsistent germination of switchgrass seeds noted in this and other studies (Aho et al., 1989; Panciera et al., 1987; Zarnstorff et al., 1994) can be attributed to switchgrass ecotypes possessing different levels of innate dormancy. Variability has also been noted between seed lots of a single ecotype produced under different environmental conditions (Panciera et al., 1987; Zarnstorff et al., 1994). In this study, both seed sources, although located in PMC fields that were spatially separated to provide required isolation distance, would have been exposed to virtually identical environmental conditions during the seed maturation period of each harvest year. However, environmental conditions varied between seed production years and, for Alamo, dormancy differences were reflected in the increased initial germination percentage of seeds harvested in 1996, when adequate rainfall and moderate temperatures occurred prior to harvest (Table 1). No such environmental influence was shown for the more highly dormant 746 ecotype.

Environmental conditions during storage had a substantial effect on germination of 746 but not Alamo, due to its reduced levels of innate dormancy and the relative rapidity at which dormancy was overcome. Seeds of 746 harvested in the fall and stored, according to standard agronomic practice, dry in either a cooler or an unheated warehouse would be expected to retain a high level of dormancy that would affect establishment if they were to be planted the following spring. Zhang and Maun (1989) found that germination of dormant switchgrass seeds did not begin to increase until >26 wk in dry storage. In this study, NC 746 seeds in low-temperature storage retained much of their dormancy throughout the entire 11-mo study period. Haynes et al. (1997) found that dormancy was not broken until after 32 mo of dry storage. Producers who store seeds under cool, dry conditions might find that long storage periods required to relieve dormancy of seed lots with a high level of innate dormancy could seriously reduce profits that could be realized from sale of these seeds.

Alternative methods may be required to improve germinability of highly dormant switchgrass seed lots. Seeds could be stored in a heated building to increase the rate at which dormancy is overcome; however, this would increase production costs, and there might be a potential pitfall to utilizing this storage method. Once dormancy is broken, viability of some seed lots has been shown to decline with further storage at high temperatures (Zarnstorff et al., 1994). Although in this study, neither 746 nor Alamo appeared to exhibit marked germination declines at the longest storage duration in the R environment, because only two years of seed production were used, it would be premature to assume that all seed lots of these two ecotypes would respond in a similar manner. Zarnstorff et al. (1994) recommended a 90-d storage period at 23 °C and, according to this study, this would appear to be an acceptable period for seeds stored at room temperature (approximately 21 °C). Seeds could then either be planted or placed in cool, dry conditions for longer-term storage.

Seeds could also be prechilled before planting. It has been demonstrated that switchgrass seeds can be prechilled, then dried and stored prior to planting without negating the effect of the prechilling treatment (Ellmore et al., 1986). The ability to dry seeds after treatment would simplify handling for both the seed producer as well as the eventual grower; however, prechilling may not improve field establishment to the same degree as it improves germination under laboratory conditions (Shaidaee et al., 1969; Zarnstorff et al., 1994). An alternative solution for planting seed lots exhibiting a higher degree of dormancy would be to provide for a natural prechilling treatment by planting early in the spring, when soil temperatures are low and moisture levels are high (Panciera et al., 1987; Sanderson et al., 1996). Planting at this time might also allow the seedlings to become established before weed pressure intensifies later in the season (Panciera et al., 1987). However, early planting may be difficult for a majority of growers in the southeastern USA, because field activities are often hampered by extremely wet soils.

CONCLUSIONS

It may not be possible to develop a single blanket recommendation on seed handling and storage conditions that would ensure optimum performance of all switchgrass ecotypes and seed lots. One approach might be to develop different recommendations based upon whether an individual seed lot exhibits a high degree of innate dormancy. Further testing using additional seed lots may be required to confirm that the results of this study apply to other ecotypes and production locations, but it appears that relative dormancy levels can be determined simply by conducting a germination test using fresh seeds. More fully dormant lots can be handled using the most acceptable of the treatment methods listed above (i.e. high temperature storage or prechilling). Seed lots with low innate dormancy will not benefit and viability could potentially be harmed by such treatments and these are therefore best stored cool and dry. Although separately treating seed lots with various dormancy levels in this manner may go far in ensuring better germination, it may not completely remedy the switchgrass establishment problem. Assuming that dormancy in a particular seed has been overcome prior to planting, environmental conditions after planting, weed pressure, and a myriad of other factors are major determinants of whether that seedling can successfully become established.

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