Rule Change Proposal No. 7

PROPOSAL: To remove the "Fresh and Dormant instructions" from "Additional Directions" for *Panicum virgatum* (Switchgrass) in Table 3. Specify a pre-germination tetrazolium test as the method to determine seed dormancy.

PRESENT RULE

Kind of Seed	Substrata	Temperature ˚C	First Count Days	Final Count Days	Additional Directions
Panicum virgatum switchgrass	P,TS	15-30	7	14	Light; KNO_3. Fresh and dormant: Prechill at 5˚C for 2 weeks. Ungerminated seeds: see sec. 4.2e and 4.9k.

PROPOSED RULE

Kind of Seed	Substrata	Temperature ˚C	First Count Days	Final Count Days	Additional Directions
Panicum virgatum switchgrass	P,TS	15-30	7	14	Light (see Sec. 4.8s); Pre – germination TZ on 200 seeds (see Sec. 4.8s).

4.7 Calculation of Percentage Germination
e. For *Panicum virgatum* (switchgrass) report results of 400 seed germination as percent germination. If the percent viable seed from the 200 seed tetrazolium (TZ) test is greater than the 400 seed germination percentage, subtract the germination result from TZ result and report the difference as percent dormant seed.

4.8 s. **Switchgrass** (*Panicum virgatum*). - Two test methods as prescribed in Table 3 shall be used on each sample. Conduct a 200 seed pre-germination tetrazolium (TZ) test as prescribed in 4.9 k. (2c), record result as percent viable seed. Place 400 seed on blotters moistened with water and germinate for 14 days at 15-30^{o} C in light, record result as percent germination. Refer to Sec. 4.7e for calculation and reporting results.

Note: if proposal 6 is adopted both species will appear in sections 4.7e and 4.8s.

HARMONIZATION: The AOSA Rules and Federal Seed Act (FSA) methods are in harmony with each other. International Seed Testing Association Rules have a 28 day germination period compared to 14 days for AOSA and FSA. Canadian Method and Procedures do not specify methods for this species.

SUPPORTING EVIDENCE: Switchgrass is a native indeterminate flowering warm-season grass which produces varying fruit sizes (seed units), some of which are commonly dormant at harvest. This dormancy dissipates over one to three years, resulting in slow establishment of field plantings (Coukos 1944 and Byers 1973). It is apparent that seed dormancy of warm-season grasses exists at normal planting dates so the use of “Fresh and dormant” treatments in seed testing laboratories has been questioned. Warm-season grasses are normally planted into warm soil conditions (20-25˚C), compared to cool-season grasses, which can be planted in fall (dormant) or spring seedings. Most warm-season grasses are sold on a Pure Live Seed (PLS) basis, which includes germinated and dormant seeds. Therefore, breaking dormancy in the laboratory is not important in the pricing of these seeds, nor does it necessarily represent the expected field emergence potential of the seed lot. With common use of TZ testing on a number of native species, the practice of prechilling to aid in determining total viability of the seed lot is of less importance.

An emerging use of Switchgrass is as a source of renewable biomass fuel production, especially due to its high forage yielding characteristic. However, the economics of producing biomass fuel requires full stand establishment in the first year of production, selecting seedlots, which are non-dormant is critical to establishing full stands of Switchgrass. The present dormancy breaking specifications within the AOSA germination methodology creates a situation where the dormancy level of Switchgrass seed lots is not clear. This situation is causing biomass producers to look for other methods such as seedlings/gram of seed as benchmarks to use in determining Switchgrass seeding rates (Vogel 2002).

Tetrazolium is widely used for checking the viability of Switchgrass seed lots and is also commonly used to check the viability of ungerminated seeds. Listing tetrazolium staining as the method for determining dormancy of Switchgrass promotes testing standardization by reducing variation caused by allowing several viability determination options.

The proposed change is supported by the Native Seed Working Group’s study of ten seed lots during the Summer/Fall of 2002. The Native Seed Working Group is composed of 14 different laboratories involved in testing native seeds, seven of these laboratories expressed interested in conducting these native grass studies. Four laboratories received seed from ten seed lots, the experimental design and data forms, three laboratories returned data (National Center for Genetic Resources Preservation, Minnesota Department of Agriculture Seed Laboratory, and Mid-West Seed Service, Inc). Testing was conducted using four true replicates of 100 seeds grouped into four separate blocks, each block containing only one of each seed lot and treatment combinations. Responses of ten switchgrass seed lots were evaluated across three prechill methods (0, 7 and 14 days of prechill). A second study evaluated three viability determination test methods across ten seed lots: 1) 400 seed TZ plus a 400 seed 14 day germination (400 pre-germ TZ), 2) 200 seed TZ plus a 400 seed 14 day germination (200 pre-germ TZ) and 3) 400 seed 14 day germination with TZ on remaining ungerminated seed (post-germ TZ) and two moistening agents water and 0.2% KNO_3. Data was collected, submitted and statistically analyzed by Amanda Patin, Mid-West Seed Services, Inc.

Prechill test results are presented in Table 1. First count germination was significantly lower without prechill (21%) compared to 70 and 74% for 7 and 14 days of prechill, respectively. Germination at 14 days was 66%, 80% and 80% for 0, 7 and 14 days of prechill, respectively. Dormant seed percentage with 0 days of prechill was 11 and 12% higher than the 7 and 14 day prechill treatment, respectively. Prechill viability means showed seven and fourteen days of prechill as providing a Post-TZ viability of 82% (Table 1). Zero days of prechill provided a post-TZ viability 79%. Pre-TZ viability was 85%.

Table 1. Mean germination, dormant seed and viable seed response of three prechill methods averaged across two laboratories, two moistening agents and ten seed lots.
N= 480

Mean Percentages

Prechill	7d germ	14d germ	Dormant	Post-TZ Total Viable	Pre-TZ Total Viable (N=80)
0 days	21	66	13	79	85
7 days	70	80	2	82	85
14 days	74	80	1	82	85
LSD	1.144	1.154	0.5177	1.128	

Significant viability differences are demonstrated between laboratories for the prechill methods averaged across ten seed lots and two moistening agents (Table 2). Laboratory one had 11% and 3% lower seed lot viabilities on 0 and 14 day prechill than laboratory two, respectively. No significant differences were seen between the two laboratories for 7- day prechill viabilities.

Table 2. Total mean viable response of two laboratories averaged across ten seed lots and two moistening agents for three prechill methods. N=160

Mean Percent Viable

Lab	0 days Prechill	7 days Prechill	14 days Prechill
1	73	82	80
2	84	83	83
LSD	1.902	1.431	1.757

Moistening agent means were significantly higher for water compared to KNO_3 across all responses (Table 3). These data suggest that KNO_3 may not be important for breaking dormancy in switchgrass.

Table 3. Mean germination, dormant seed and viable seed response of two moistening agents and ten seed lots across three laboratories. N=720

Mean Percentages

Moistening Agent	7d germ	14d germ	Dormant	Viable
Distilled H_20	43	69	16	85
0.2% $KN0_3$	33	63	21	84
LSD	0.8684	0.7908	0.9658	0.7271

Three laboratories returned results for the viability method determination portion of this study and their respective germination test responses for 240 observations are presented in Table 4. Viability of the ten seed lots ranged between 60-96%, 53-97% and 52-95% for the 400 pre-germ

TZ, 200 pre-germ TZ and post-germ TZ, respectively. These Switchgrass seed lots are representative of what laboratories could expect to see submitted to their laboratories.

Table 4. Total mean viability response from ten seed lots for 400 pre-germ TZ, 200 pre-germ TZ and post-germ TZ from three laboratories averaged across two moistening agents. N=240

Mean Percent Viable

Seed Lot	400 pre-germ TZ*	200 pre-germ TZ**	Post-germ TZ
1	92	90	89
2	60	53	52
3	88	86	84
4	66	74	68
5	89	88	90
6	85	82	81
7	95	97	95
8	96	95	90
9	94	95	88
10	89	91	91
LSD	2.985	5.121	2.462

* N=120 ** N=60

Fourteen-day germination percentages were identical for all treatments, while total viability decreased by 2% for post-germ TZ when compared to 400 pre-germ TZ and 200 pre-germ TZ (Table 5). Note: the germination data for 400 and 200 pre-germ TZ methods utilizes the same seedling sets.

Table 5. Total mean viable response averaged across three laboratories, ten seed lots and two moistening agents for 400 pre-germ TZ, 200 pre-germ TZ and post-germ TZ. N=720

Mean Percentages

Method	7d germ	14d germ	Dormant	Total Viable
400 pre-germ TZ	39	66	19	85
200 pre-germ TZ	39	66	19	85
Post-germ TZ	37	66	17	83
LSD	1.287	1.332	1.422	0.7028

Significant differences are demonstrated between laboratories for the three TZ methods averaged across ten seed lots and two moistening agents (Table 6). Six percent differences are shown for 400 pre-germ TZ and 200 pre-germ TZ, while for the post-germ TZ a three percent difference was seen between the three laboratories.

Table 6. Total mean viable response of 400 pre-germ TZ, 200 pre-germ TZ and post-germ TZ for three laboratories averaged across ten seed lots and two moistening agents for. N=240

Mean Percent Viable

Lab	400 pre-germ TZ*	200 pre-germ TZ**	Post-germ TZ
1	89	88	84
2	85	85	84
3	83	82	81
LSD	1.635	2.805	1.348

* N=120 **N=60

LITERATURE CITED

Byer, K.L. 1973. Evaluation of methods of reducing seed dormancy in switchgrass, indiangrass, and big bluestem. Thesis South Dakota State University. 40p.

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