

A newsletter for members of AOSA/SCST

### Seed Technologists Newsletter



Volume 90, Issue 2

November, 2023

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"Nature does not hurry, yet everything is accomplished." -Lao Tzu



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## Newsletter Submission Guidelines

Communications & Publications Committee

Articles should be typed, pertaining to some aspect of seed testing or other items of interest to the AOSA and SCST membership. These may include, but are not limited to:

- Ongoing research
- Committee and Working Group activity
- Updates on the financial state of the organizations
- Distinguished member profiles
- Profiles of new members to the organizations.
- Research paper abstracts
- Results of research, referees, and validation studies
- Upcoming changes to the AOSA Rules
- Upcoming changes to the By-Laws of either organization
- Survey study results
- Information from other seed-trade organizations
- Regional updates to state seed laws or RUSSL
- Information on upcoming workshops or other opportunities for training
- Book and resource reviews
- Impressions from the Annual Meeting

Formatting:

- Please include images as **separate** files, with credit to the photographer if different than the author. All images used will be credited.
- For specific formatting within a document, please do not insert images, but leave a placeholder so that the editorial staff can include appropriate images, graphics, and tables within articles.
- Please do not submit PDFs of articles.

Citations:

- Cite image sources and references used.
- Cite any additional sources used to compose the article, including co-authors so that they may be credited.
- Author's name and contact information to be included in our contributor's page.

Publications must be in accordance with the Anti-trust policy of the AOSA and SCST.



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## **Calendar of Events**

Euroseeds Annual Congress & Meeting	October 14-18, 2023	St. Julians, Malta
ASTA Western Seed Conference	November 1, 2023	Kansas City, MO
Seeds Canada - Semi-Annual Meeting	November, 17-20	Virtual
Corn Belt Conference	November 14-16, 2023	Indianapolis, IN
Asian Seed Congress	November 20-24, 2023	Christchurch, NZ
ASTA Field Crop Seed Convention	December 5-8, 2023	Orlando, FL
OSA Winter workshop, Winter meeting	January 18-19, 2024	Lebanon, OR
ASTA Vegetable & Flower Seed Conference	January 26-30, 2024	Monterey, CA
OECD Seed Schemes Working Group	January 29-February 2, 2024	Livingstone, Zambia
SCST Genetic Technology Superworkshop	February 5-9, 2024	Iowa State University, Ames IA
California Seed Association Annual Convention	March 17-20, 2024	Coronado Bay, CA
AOSA-SCST Annual Meeting	June 2-6, 2024	Rapid City, SD
ASTA Leadership Conference	June 15-19, 2024	Nashville, TN



Upcoming project updates from committee chairs

#### **Communications & Publications Committee**

Seed Technologists Newsletter -

The Newsletter is looking for submissions, particularly research abstracts and presentations, resource reviews, and questions and photos for the study guide section. Submissions to the Newsletter can be made by emailing the editors, Kathryn McGinnis and Quinn Gillespie, or by submitting via <u>Google form</u> on the Communications and Publications Committee website.

Historical Documents -

The committee is working through historical documents to make a database of topics. Volunteers are encouraged and welcome! There is a <u>Google form</u> available to enter data and the results can be viewed as a <u>Google sheet</u>. Special thanks to Heidi Larson, Neal Foster, and Brent Reschly who have worked to scan so many of our historical documents. Now we want to make them searchable!

#### **Continuing Education Committee**



- The new point accumulation period is June 1, 2021 to May 31, 2024

- Shortage notices will be sent early in 2024

- Any questions?

AOSA: CEpoints@scda.sc.gov

SCST: katherine.mathiason@sdstate.edu



Upcoming project updates from committee chairs

#### **Genetic Technology**

The Genetic Technology committee met virtually via zoom to begin work for the 2023-2024 year. The Genetic Technology Committee has established four subcommittees to focus on writing committee SOPs for the master list of documents, Education, Newsletter, and Research. Committee members may sign up for subcommittees or may be assigned a subcommittee based on need.

The SOPs subcommittee, headed by Kalyn Brix, will be focusing on getting committee documents finalized for the Lab Standards and Documentation Committee by January 1, 2024. The Education subcommittee, headed by Molly Richeson, is focused on the 2024 Genetics Superworkshop, to be held at Iowa State University. The current tentative dates are February 5, 2024 - February 9, 2024. The Newsletter committee, headed by Molly Richeson, will be working on new member profiles for members who passed their CGT/RGT exams, and on developing a study quiz for the membership. The research subcommittee, headed by Kalyn Brix, is focused on developing a list of study ideas, with a comparison between in-person and virtual HB evaluations proposed as one topic, and AP results between labs suggested as a second topic.

The Genetic Technology Committee plans to meet monthly to accomplish these goals.

#### Mark Your Calendar for the 2024 SCST Genetic Testing Super Workshop!

Please watch for emails and check Analyzeseeds.com for details for the 2024 SCST Genetic Testing Super Workshop which will be held February 12-16 at Iowa State University Seed Science in Ames, Iowa. The upcoming week of training will continue the tradition that first started in 2004 of hands-on training, lectures and networking about genetic testing. Genetic testing includes checking for biotech traits in seeds, intended or unintended and also genetic (cultivar) purity testing. Some of the general areas covered include basics of molecular biology, herbicide bioassay, ELISA, lateral flow strips, electrophoresis and DNA-based testing. There are good reasons to come to Iowa in February! Please contact Molly Richeson (molly.richeson@agreliantgenetics.com) or Mike Stahr (mgstahr@iastate.edu) for more information.

#### Handbook Committee

The Handbook Committee is seeking active members to participate in editing handbooks for the organization. New members of the committee will have the opportunity to be trained in the process of incorporating updates and proofreading handbooks.



Upcoming project updates from committee chairs

#### **Purity Committee**

The Purity Committee is actively looking for new members. The committee is currently drafting two rule proposals for 2024. One proposal is to clarify the reporting requirements for contaminants found in the purity. The other proposal is to add hybrid bromegrass, *Bromus riparius x B. inermis* to the AOSA Rules Volume 3. This species would be classified as an Agricultural crop.

At the committee meeting during the Annual Meeting the committee chairs also asked for volunteers to help look through the AOSA rules any conflicts within the rules, or points of clarification that might be needed to improve the purity analysis chapters. The chairs also asked for volunteers to check common names in the AOSA rules against the Federal Seed Act, GRIN, and other published plant databases to ensure the Rules are using the most current common names.

#### Genetic Technology Proficency - by Lauren Shearer

Every year a written proficiency is sent out to the members that covers genetic technology with a focus on either Adventitious Presence, Genetic Purity, or Trait Confirmation. All RGT, CGT, and professional members (with genetic certification) are required to participate, while associate members are not required but encouraged to participate. For the 2023 SCST Adventitious Presence (AP) written proficiency there were 27 participants (15 RGT, 9 CGT, 2 Professional, and 1 Associate). All participants passed (needed a score higher than 80%) and the average score was 92%. For the nineteen multiple choice/True or False questions, all participants correctly answered question 2, 6, 9, 14, 15, and 16. Question 13 was the most challenging, with only 43% of the respondents answering correctly, while the remaining questions were answered correctly by 75-96% of the participants. Question 20 was a "gimme" as participants were awarded points regardless of answer. All participants answered questions 21-25 correctly, which focused on the use of SeedCalc8.

Can you answer question 13? "It is very important that all dNTP concentrations are equal to prevent denaturing of bases. True or False?

False. It is very important that all dNTP concentrations are equal to prevent misincorporation of bases.



Upcoming project updates from committee chairs

#### **Referee Committee**

Region 1: Rachel Henricks is seeking participants for a study on Balansa clover (Trifolium michelianum) to test germination methods. Seed count data is also welcome. Marija Topic is also conducting a study on sweet corn and has already sent out samples to six different labs.

Organization-wide referee: Basil germination, to add TB to the methods for Basil. Coordinated by Nicolette Hard and Quinn Gillespie. To participate please contact Nicolette Hard.

Region 6: Canada has a new regional co-chair, Dr. Lei Ren from CFIA. Canada has several projects comparing germination methods between AOSA, ISTA, and Canada in the works, including orchardgrass, Kentucky bluegrass, and chickpea.

The committee is also still evaluating potential new categories for referees and validation studies and is in the process of updating the referee project guidelines.

#### **SCST Constitution and Bylaws**

After this round of changes to the Constitution and Bylaws, Tom Mager will be taking over as chair of the SCST Constitution and Bylaws Committee. The changeform on the website has been updated to include his email. Any proposed changes to the SCST Constitution and Bylaws should be sent to the new chair, or the Executive Board.

SCST Constitution and Bylaws Proposed Changes:

- Examination requirements for the reinstatement of Registered and Certified Members Inactive
- Reduced time requirement for Associate members before they are eligible for the exam
- Specification for determining candidate's time as an associate member.
- Passing requirements for accredited courses used for points toward exam eligibility
- Constitution and Bylaws amendment procedure.
- Voting on AOSA Rules
- Distribution of representation on the Board of Directors.

The full proposed text for these items is posted on the Constitution and Bylaws Page. Please submit your online ballot via the SurveyMonkey link sent by the SCST Administrative Office by October 25, 2023. If you do not have the link, please contact Kelly Polzin for a ballot before October 25, 2023.

## Call for Submissions to SIG

#### Quinn Gillespie, RST



ISMA is seeking author submissions and photos for their Seed Identification Guide Fact Sheets! This is part of the AOSA/SCST/ISMA joint working group project: The Development of Digital Reference for Seed Identification of the species on the AOSA/SCST Exam List. Fact sheets will be added to the Seed Identification Guide, a published, peer-reviewed virtual book. (ISBN: 978-1-7753419-0-1). Authors of fact sheets will be compensated with \$100 per completed approved fact sheet. Photographers of seeds will be compensated with \$100 per completed set of photos for fact sheets. Analysts do not have to complete both portions in order to submit their fact sheets. If you do not have good imaging technology available, analysts can still complete the fact sheets to aid others in seed identification. Help sourcing literature is also available from ISMA. There is a lot of guidance available to analysts to get these fact sheets completed. (See Sidebar)

The current goal is to have 100 species added in the next two years. This is an ambitious goal, but one that we are capable of tackling as a group. The more people contributing their expertise to this project, the more useful and comprehensive this tool can be for many years to come! Fact Sheet Resources SIG Publication Guide Author Workshop & Webinar Descriptions of fruit types Seed Size Measurement Protocol Seed Shape Chart Surface Feature Comparison Chart Seed Color Chart Common Embryo Descriptions Seed Imaging Equipment

General guidelines for fact sheets:

- □ Use plain English words when describing seeds. Remember the end user may be international or may not have training in botany.
- Use standard descriptors for color, shape, and size. See the sidebar for links to these resource pages.
- □ Features of dispersal units should be typical of the species but may include observations on the range of natural variation.
- □ Common crops must be listed first by botanical name, followed by common name for the first reference. Afterward they may be referred to by common name.
- □ Wild species or uncommon crops must be referred to by botanical name.
- Review reliable sources for current common and botanical names used to refer to any species, such as GRIN, GBIF, ITIS, etc. A list of approved sources is provided on the <u>SIG Publications Guide</u>.

If you are interested in contributing to the Seed Identification Guide, either in the form of writing fact sheets or providing seed images please contact <u>Kathy Mathiason</u>, <u>Marija Topic</u>, or <u>Ruojing Wang</u>.

### Abstract: Effect of wet and dry ZnO, TiO2, SiO2 based nano-particles on physiological and biochemical parameters of Chickpea (Cicer arietinum) seeds

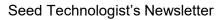
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#### ABSTRACT

Explosive demand of higher agricultural productivity to feed the increasing population demands innovative technologies to improve the seed quality in order to harness the full potential of genetic resource and other agronomic inputs. The use of nano-based technology for seed treatment has a potential to upgrade the seed quality with minimalistic exposure to chemicals making it ecologically and economically sustainable. The present study is an attempt to assess the prospects of nanotechnology as an alternative approach to improve the seed quality. The seeds of chickpea variety Pusa-547 were treated using zinc, silicon, and titanium oxides in various combinations (viz., 50, 100, 250, 500 and 750 ppm). The observations for various seed quality parameters were recorded to find out the best treatment that could improve the physiological and biochemical attributes of seed. Amongst the various treatments given to the seeds of the chickpea variety Pusa-547, none of the treatment were significantly superior than control. The treatment Dry Bulk ZnO @500 ppm recorded significantly highest values for most of seed quality which was at par with control.

Keywords: Seed, Nano, Agriculture



### Abstract: The Seedling Evaluation Database Model: Proposing Changes to Evaluation Rule

Dr. Riad Baalbaki, Senior Seed Botanist CDFA

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Seedling evaluation descriptions in Vol. 4 of the AOSA Rules are difficult to develop for new species and to modify for existing ones. The low number of major changes to Vol. 4 in the past years reflects this difficulty. Referee tests are not feasible since development and types of abnormals are almost impossible to predict when testing samples using multiple lots among several labs. Moreover, determining what constitutes normal and abnormal development cannot be decided by a 'popular' vote, yet empirical determinations are not feasible and only possible following large-scale experiments. In addition, many existing descriptions, such as 'short hypocotyl,' involve a subjective determination by analysts, and can vary according to training and familiarity with the species. A model like the one developed to determine the correct evaluations of pictures added to the Seedling Evaluation Database is suggested. The model is based on three components: a) surveys of a large number of analysts on different types of developmental conditions; b) data analysis exploring dependance of evaluations on level of analyst expertise and differences within each experience level; and 3) extrapolation of the results and analysis to propose normal/abnormal evaluations per species or family and guidelines for quantitative measures to replace subjective descriptions. A general description of this model with examples based on actual survey data is presented.



### Abstract: An investigation of seed longevity and antioxidant changes of conserved barley germplasm in Plant Gene Resources of Canada

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It is challenging to maintain seed viability above a predefined threshold like 80% of initial viability (P80) for germplasm preserved in genebanks under low moisture content and low temperature, as regular viability assessments of the conserved seeds are largely inaccurate. This research attempted to predict the longevity of barley (Hordeum vulgare ssp. vulgare & Hordeum vulgare ssp. spontaneum) germplasm preserved in PGRC long-term storage using seed viability models and seedling length data and to compare the intra-specific differences in seed longevity of barley accessions using viability curves and antioxidant parameters. A diverse sample of covered and hulless barley accessions, including different improvement groups namely cultivars, breeding lines, genetic stocks, landraces, and wild relatives, were assayed for seed viability and seedling length. The viability loss of the barley accessions was well explained by non-linear Weibull type I- three parameter models. Differentiated viability loss patterns were observed between covered and hulless accessions and different germplasm subtypes within covered barley. Hulless and covered barley accessions preserved at  $-20^{\circ}$ C reached P80 within 36 and 39 years of storage, respectively, followed by the more rapid decline of viability for hulless barley. The seedling length was reduced with increased storage time and suggested their rapid onset of viability loss around 31 - 36 years. Also, the longevity predictions based on Transformed Coefficient of Variation (TCV) of viability seemed to be more realistic than that of TCV of seedling length. A decline in DPPH and FRAP antioxidant activities was observed for covered breeding lines, but the phenolic content of these accessions increased regardless of seed age. However, no clear pattern of antioxidant parameter change over storage time was observed within the covered cultivars and wild relatives. These findings together suggest that inferring longevity parameters at intra-specific levels may be useful for better management of seed collections in genebanks.

Keywords: Barley, Seed Genebank, Long-term storage, Longevity prediction, Antioxidant activity

*Abbreviations:* DPPH: 1,1-diphenyl-2-picryhydrazyl; FRAP: Ferric reducing antioxidant power; PGRC: Plant Gene Resources of Canada; P80: 80% of Initial Viability; TCV: Transformed Coefficient of Variation



### Abstract: Weed seeds detection using a mechanical auto-shaker

Solomon Sakyi-Quartey, Yusuf Abuke, Ruojing Wang\*, Yimeng Wang

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Retrieving weed seeds from a testing sample is usually conducted through a manual procedure with or without the assistance of sieves. The manual method is time-consuming and ergonomically challenging. The auto Sieve Shaker equipment operates under a set of parameters that includes stacked sieves (same diameter, different pore sizes), time durations, and controlled frequencies. The application of the equipment potentially could achieve efficient, reliable, and consistent outcomes. Our research team studied the pilot application of the auto Sieve Shaker in the mechanical separation of targeted weed species from seed samples during a search for noxious weed seeds. The weed seeds targeted included Canada thistle (*Cirsium arvense*) and *Cuscuta* species in samples of Pea (*Pisum Sativum*), wheat (*Triticum aestivum*), barley (Hordeum Vulgare), flax (Linum usitatissimum), canola (Brassica napus var napus) with two levels of sample contamination. We defined contamination level as average when foreign materials were < 0.9%and dirty samples when foreign materials were > 1% and near 3%. The targeted weed species and their similar species were spiked into these samples. The parameters for auto shaking, such as shaking frequency and duration, and appropriate sieve size were tested for different crops and samples. The outcome demonstrated 100% retrieval rate can be achieved for the detection seeds of Canada thistle with auto shaking frequency of 0.35-0.5 g for wheat and 0.5-0.75g for barley in 3 minutes shaking duration. Retrieving Cuscuta species with 100% accuracy with 0.75g 3 minutes for canola and pea, but 5 minutes for wheat and flax. The auto Sieve Shaker is an efficient and reliable solution for separating weed seeds. By selecting the right sieve sizes and operational parameters, such as shaking frequency and duration, tailored to each crop and target species, the auto shaker enables high-throughput weed seed detection. This method is particularly effective for small-seeded contaminants like Cuscuta and improves laboratory efficiency while reducing costs associated with training and ergonomic damage. Overall, the auto Sieve Shaker will potentially offer a time-efficient and consistent alternative to manual seed separation, enhancing accuracy and efficiency for noxious weed search.

# Impact of particle size of ground corn samples on the detection of the presence of 35S and TNOS sequences.

Lauren Shearer, Kalyn Brix, presented by Kalyn Brix

Company: SoDak Labs Inc Address: 236 32 nd Ave, Brookings, SD 57006 Phone Number: 605-692-2758 Presenter email address: Kalyn.Brix@SoDakLabs.com

Accurate analysis of the adventitious presence of a genetically modified organism (GMO) is crucial for meeting certain market and export guidelines. One influence on the accuracy can be the particle grind size of the sample. Research studies of different particle grind sizes were conducted across three laboratories to provide insight on DNA detection capabilities. Event-free corn base material (EFBM) was verified by subsampling three pools of 1000 seed and screening for the detection of 35S and TNOS with real-time PCR using in-house validated methods. A spike material (SM) for corn event MON-88017 was verified on 90 individual seeds using ELISA methods for Cry3Bb protein detection. The EFBM and SM was then ground and sieved to separate the ground material into two particle sizes (<1mm and 1mm-2mm). Using the sieved material, a 0.4% and 0.9% spike level sample for each particle size were prepared on a % w/w basis for a 400 and 200 seed pool, respectively. For detection of the 0.4% spike level, each laboratory received 10 tubes of each particle size containing 1.0 gram of ground material. Eight of the ten tubes contained a subsample of the 0.4% spike level while the remaining tubes contained a subsample of the EFBM. For the detection of the 0.9% level, each laboratory received 6 tubes of each particle size containing 1.0 gram of ground material. Three of the six tubes contained a subsample of the 0.9% spike level while the remaining tubes contained a subsample of the EFBM. Participating laboratories extracted the samples for DNA analysis using their in-house validated real-time PCR methods for the detection of 35S and TNOS. Qualitative results were reported and summarized by SoDak Labs. Based on the dataset, neither particle size impacted detection for TNOS at the 0.9% level for any of the laboratories, nor for 35S at the 0.9% level for two of the labs. For both particle sizes, a false positive was detected for 35S at the 0.9% level for one of the laboratories, however it was commented Cq values were close to negative threshold. At the 0.4% level, a false positive was detected for the <1mm particle size by one laboratory. Both laboratories correctly identified negative subsamples at the 1mm-2mm spike level. The limit of detections was reported at 0.03% and 0.01%. This research was funded in part by a grant from the Seed Testing Research Foundation. Thank you to AgReliant Genetics, LLC and 2020 Seed Labs Inc. for their participation.

# Dormancy Breaking Methods in Freshly Harvested Oilseed Sunflowers

Miranda Smidt, Lauren Shearer, Tim Gutormson

#### April, 2023

#### Introduction:

The Association of Official Seed Analysts (AOSA) "Rules for Testing Seeds" lists multiple methods to break dormancy on freshly harvested seeds. One method cited in Chapter 6.9h is for two rates of ethephon: standard rate of 0.0029% (29 ppm) and a concentrated rate of 0.0145% (145 ppm). In Table 6A *Helianthus maximilliani* references section 6.2f (Light) and 6.9m (Viability testing of ungerminated seed) with its known dormancy but has no reference to dormancy breaking methods. For *Helianthus annuus*, no dormancy breaking method is specified in Table 6A; the objective of this study is to identify an effective and consistent method to overcome primary dormancy in freshly harvested hybrid oil seed sunflowers. Primary dormancy can lead to questionable preliminary viability evaluations and uncertainty that the seed viability will meet production contract specifications. Typically, within 2-3 months post-harvest, after ripening diminishes the remaining primary dormancy in the seed population, producing seed germinations that meet the production standards. Confirming a method that effectively breaks dormancy would provide a streamlined test that can forego 6.9m, and further allow processing, conditioning, and packaging a freshly harvested hybrid oil seed sunflower lot in a timely manner. Findings of this study could lead to a proposal on a *Helianthus annuus*, specifically hybrid oil seed sunflowers.

#### Literature Review:

Dormancy in seeds can be expressed in exogenous and endogenous types and are imposed morphologically, physically, or physiologically. Hybrid oil seed sunflowers can produce a normal plant from embryo twenty-one days after anthesis, and onset of dormancy happens thereafter, so it can be concluded that they do not experience morphological dormancy. To break the physical and physiological types of dormancies, methods such as growth regulators, pretreatments, mechanical scarification, and combinations thereof have been evaluated.

The physical barrier of a sunflower embryo includes its hardened pericarp and the integument/testa maternal layer. Pretreatments are a commonly used method in initiating germination for dormant species. In sunflowers, pretreatments have ranged from dry heat treatment (ranging: 60°C for 15 minutes to 100°C for 5 minutes) [6, 12], water soak (ranging: 25°C for 15 minutes to 100°C for 30 minutes) [1, 4, 7, 8, 10], pre-chill (ranging 5-10°C) [15], freeze/thaw cycling (-80°C to 20°C cycling) [8], microwaving (80% for 30 seconds to 100% for 60 seconds) [12], smoking (sambrani for 3 hours) [12], seed chipping [3, 4, 8], and complete removal of the pericarp and maternal layer [13].

Physiological inhibitors are a balance of the hormones within the sunflower seed. Seed chemicals, namely that of abscisic acid (ABA) and gibberellic acid (GA<sub>3</sub>), are a delicate balance that trigger the seed when to initiate germination. Induction and maintenance of dormancy is regulated by ABA, while germination is enhanced by GA<sub>3</sub>. Growth regulators are then sought out to offset the balance of ABA and initiate germination for dormant species. GA<sub>3</sub> applications (ranging from 0.01% -0.1% or 100ppm-1000ppm) have been evaluated for overcoming the ABA inhibition [3, 4, 12, 15]. Various other chemical treatments have also been investigated, such as the efficacy of thiourea (0.01%-0.5%) [10, 12], Ethrel, whose active ingredient is ethephon (0.0005%-0.3%) [6, 9, 10, 12], hydrogen peroxide (5 minutes) [3], ethanol (25% for 15-30 minutes) [9, 10], acetone (25% for 15 minutes) [6, 9, 10], 1-aminocyclopropane-1-carboxylic acid (ACC) (ranging 0.0000001%-0.01%) [11], and ethylene gas (20 hours at saturated atmosphere) [8].

The challenge proposed to the seed testing industry is finding a quick and effective method for breaking dormancy in freshly harvested hybrid oil seed sunflowers. We propose bringing a new angle to breaking dormancy with three new methods: 1.) Lengthening the preheat dry time to 3d @  $30^{\circ}$ C, 2.) Lengthening preheat dry time to 7d @  $30^{\circ}$ C, as well as 3.) Introducing a lengthened preconditioned stratification where seeds are exposed to -20°C conditions for 7d and further submerged for 18 hours in a solution of 0.05% (500 ppm) and 0.025% (250 ppm) of GA<sub>3</sub> and ethephon respectively prior to roll towel planting.



Then comparing this to previous tried methods: 1.) 18-hour 0.05% GA<sub>3</sub> soak [4] 2.) 18-hour 0.025% Ethrel soak 3.) Utilizing 0.05% GA<sub>3</sub> as a blotter moistening agent, and 4.) Soaking the seeds in tap water for 18 hours and clipping the cotyledons prior to planting. These will be compared against a roll towel test with tap water as the blotter moistening agent.

#### **Materials and Methods:**

SoDak Labs, Inc. received 10 seed lots of freshly harvested (September 2022) hybrid oil seed sunflowers *Helianthus annuus* (contributed by RemSun Inc. and Syngenta Seeds Inc., Arbuckle and Glen, California, respectively). Upon receival, all seed lots were subjected to an electronic seed moisture reading using a Steinlite SL95 Moisture Meter. Seed was then screened to remove inert matter and sized over three round screens (16/64", 14/64", and 11/64") to create two working seed sizes. Any seed over the 16/64" and under the 11/64" screen was discarded and not included in this study. Seed below the 16/64" and above the 14/64" was classified as 'size 2,' while seed below the 14/64" and above the 11/64" was classified as 'size 3.'

A Tetrazolium (TZ) test of two (2) replicates comprised of 100 seed per size per lot was conducted according to the prescribed methods in the Tetrazolium Testing Handbook published by AOSA and SCST (2010 edition) to establish maximum viability of each respective seed lot and size. Germination and dormancy breaking treatments were determined using four (4) replicates of 50 seeds per size per seed lot. All germination tests were conducted using the rolled towel method on 38#, 12x24 cm brown paper towels (Anchor Paper Company, St. Paul, MN) and completed within 30-45 days of harvest. These towels were positioned upright and enclosed in plastic bags to prevent towel drying and were incubated at 20°C for 7 days. Evaluation occurred on day 7 and classified the seedlings as normal seedlings, abnormal seedlings, dead and firm seedlings in accordance with the AOSA Seedling Evaluation Handbook 'Asteraceae, Sunflower Family II – Kinds other than lettuce' [1]. 'Dead seeds' were ungerminated seeds that exhibited flaccid embryo tissue. 'Firm seeds' were classified as ungerminated seeds with firm embryo tissue at the conclusion of the test duration. (These seeds were not further investigated for viability at the termination of the test so they cannot be classified as either dead or dormant.)

Germination studies include 1.) a water check (no dormancy breaking) and the following seven seed dormancy breaking methods: 2.) heat drying pretreatment for 3d @ 30°C, 3.) heat drying pretreatment for 7d @ 30°C, 4.) 18-hour pretreatment soak in 0.05% gibberellic acid  $\geq$ 90% (Product # G7645, Sigma Aldrich) (GA<sub>3</sub>) solution 5.) 0.05% GA<sub>3</sub> as media moistening agent, 6.) 18-hour pretreatment soak in 0.025% ethephon/Ethrel (21.7%, Product # 5P95, HGI Worldwide, Inc.), 7.) stratify seed by placing on a screen for 7d @-20°C followed by a preplant treatment of an 18-hour soak in a 0.05% GA<sub>3</sub> + 0.025% Ethrel solution, and 8.) 18-hour water soak followed by clipping cotyledons. All treatments were planted with the same methodology as the germination test: using water as the blotter moistening agent unless otherwise stated. Data was analyzed using R Studio Version 1.4.1103. An ANOVA model was used to calculate treatment means significant differences with LSD at (P<0.05).

#### **Results and Discussion:**

Seed moisture levels of the 10 composite seed lots ranged from 3.51% to 5.73% (Table 1.). These are considered low seed moistures as the target range for hybrid oil seed sunflowers is 6-8% post-harvest (personal communication, Dan Howe, RemSun Inc., Arbuckle, CA). The seed moisture levels of lots 5 and 6 were 3.51% and 4.21% respectively and exhibited the highest seed dormancy levels: 53% and 51% respectively.

Viable seed percentages of the 10 seed lots averaged across the two sizes ranged from 93 to 100% indicating high freshly harvested seed quality. Post germination firm seed percentages ranged from 1 to 53%. Total viability established in the tetrazolium test, paired with the normal seedlings and firm seeds of each lot provided a basis to measure efficacy of treatment without detrimental effect on readily germinating lots; furthermore, this provided a realistic set of seed lots for the study objectives.

Normal seedling, abnormal seedling, and dead seed percentages of the seed lot set were as to be expected in freshly harvested hybrid oil sunflower seed. The abnormal percentage of seed lot 6 was 13%, significantly higher than all other seed lots. Since seed lot 6 had a considerably high level of seed dormancy, some of these abnormal seedlings may be in transition from primary dormancy to a normal seedling but had insufficient development at the time of normal seedling evaluation.

Table	Table 1. Comparison of 20°C germination normal, abnormal seedlings, dead, firm seed and viable seeds from the TZ results of ten sunflower seed lots averaged across eight treatments and two sizes.													
	Normal Seedlings	Abnormal Seedlings	Dead Seeds	Firm Seeds	Viable Seeds	Seed Moisture								

Lot						%				
1	86	с	4	de	6	a	4	f	99	5.34
2	70	f	5	cd	8	a	17	с	93	4.24
3	80	e	4	de	4	b	12	d	98	4.6
4	90	b	2	e	1	d	7	e	99	5.21
5	40	g	5	с	2	bcd	53	a	99	3.51
6	35	h	13	a	1	d	51	a	98	4.21
7	84	cd	6	с	3	bc	7	ef	94	4.16
8	94	a	3	e	2	cd	1	g	99	5.73
9	69	f	8	b	2	cd	21	b	100	4.64
10	83	de	5	cd	2	cd	10	d	99	4.19
LSD	3 1.7			1	.6	2.5		5.5	4.2	

Results of eight treatments are presented in Table 2. Treatments 6 and 7 were significantly different and higher than that of other treatments for normal seedling percentages: 91% and 89% normal seedlings, respectively. The water check was statistically significantly lower than all treatments except treatment two. Both preheating treatments (2 and 3) had minimal impact on reducing seed dormancy levels. Treatments utilizing  $GA_3$  and Ethrel (4, 5, 6, and 7) had the most significant impact on breaking sunflower seed dormancy ranging from 76% to 91% normal seedlings and 1 to 17% firm seeds. It appears that a slight synergistic effect occurs with a combination of Ethrel and  $GA_3(7)$ . It is unclear whether the synergistic effect is equivalent to a presoak or a blotter moistening agent: further comparative studies of these methods could focus on the synergistic reaction of these two growth regulators.

Abnormal seedling percentage was significantly higher in treatment 8. Clipping did reduce seed dormancy significantly compared to the water check (1) but did induce more abnormal seedlings at the time of evaluation. These seedlings were considered abnormal due to their top-down fashion of growth. Seedling cotyledons would begin to develop active chlorophyll and start photosynthesizing, while the radicle tip had shown little to no sign of developing: remaining intact and firm, with little to no elongation and dividing (Figure 1).



*Figure 1. Developing cotyledons at day 7 of clipped cotyledons treatment. Left: seedlings stuck in seed coat. Right: seedling seed coat removed.* 

Dead seed percentages were statistically different but only varied 4% between the highest (3) and lowest (8) treatments' dead seed percentages.

Table 2. Comparison of 20°C germination of normal, abnormal seedlings, dead, firm seeds for eight treatments averaged across ten seed lots and two seed sizes using freshly harvested hybrid oilseed sunflower seeds. Abnormal Trt Treatment Normal Firm Dead Seeds No. Name Seedlings Seedlings Seeds 1. Water as Blotter Moistening Agent (BMA) f с bcd 58 2 3 37 2. 3d @ 30°C pretreatment, water BMA 57 <sup>f</sup> bc bc 4 3 36 3. 7d @ 30°C pretreatment, water BMA b e а 4 6 27 63 0.05% GA<sub>3</sub> 18h presoak treatment, water 4. b bc cd BMA 84 4 2 10 5. 0.05% GA3 as BMA b bcd 76 <sup>c</sup> 4 3 17 0.025% Ethrel 18h presoak treatment, water 6. b bc **BMA** 91 а 4 4 1 7. 7d @ -20°C followed by 0.05% GA<sub>3</sub> + 0.025% f b 89 <sup>a</sup> cd Ethrel 18h presoak, water BMA 4 3 4 8. 18h presoak, Clipped Cotyledons, water BMA d d а d 67 2 18 13 LSD 2.6 1.5 1.3 2.1

Seed size responses are presented in Table 3, as there was a 1% higher overall normal seedling count in size 2 than in size 3.

Table 3. Comparison of 20°C germination of normal, abnormal seedlings, dead, firm seeds of two seed sizes averaged across ten seed lots and eight treatments for sunflower seeds. Abnormal Dead Seeds Normal Seedlings Firm Seeds Seedlings Size b а а 2 74 а 5 3 18 b b 3 5 а 3 а 73 19 LSD 0.8 0.5 0.4 0.7

Seed sizes were compared across seed treatments in Table 4. With only three significant differences between size 2 and size 3 for normal seedling percentages. The water check had significant differences in dormancy between sizes for seed lot 2 and 7; however, lot 2 exhibited higher dormancy in size 3, while size 2 was more dormant in lot 7. Seed lot 4 had significantly higher dormancy in size 3 with the preheat 7d @ 30°C treatment. It is hard to draw a consistent conclusion on whether seed size and seed dormancy are closely related based on the findings of this study.

Table 4. Comparison of warm germination percent normal seedlings of eight treatments for ten seed lots with two seed sizes for sunflower seeds.																				
Treatment	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5		Lot 6		Lot 7		Lot 8		Lot 9		Lot	10
Treatment	2	3	2	3	2	3	2	3	2	3	2	3	2	3	2	3	2	3	2	3
1	85	95	62*	43*	67	71	93	77	5	2	1	3	70*	89*	91	82	40	40	74	71
2	82	80	22	24	34	50	76	67	26	30	13	14	89	83	93	97	59	54	82	72
3	75	92	57	55	62	67	89*	66*	28	21	6	14	84	91	94	96	49	50	84	75
4	84	81	93	90	93	94	100	98	67	56	55	62	88	82	99	98	90	88	86	75
5	74	89	89	84	92	92	96	98	38	32	16	31	91	87	97	97	71	80	90	85
6	80	94	84	80	93	93	97	97	92	91	92	94	88	93	97	97	94	97	93	95
7	91	90	93	85	95	96	100	98	74	68	73	69	95	91	97	98	96	91	97	98
8	94	96	94	79	93	94	98	93	2	8	13	7	73	61	93	90	60	44	80	74
LSD <sup>1</sup>	17																			
<sup>1</sup> Significant differences between seed sizes within lot noted by "*".																				

Conclusions can be made that stratifying seed and following with a treatment of  $GA_3$  and Ethrel may have a synergistic value in reducing dormancy in freshly harvested hybrid oil seed sunflowers without negatively impacting the nondormant seed population. Ethrel and prechilling may be the most practical solutions from a cost-effective standpoint. The Ethrel rates used in this study were significantly higher than that of which is stated in the AOSA Rules [1] – at a 0.025% study rate compared to that of a 0.0145% maximum. Ethrel at the rate of 0.025% and  $GA_3$  at a rate of 0.05% should be considered as an upper bound as it resulted in significant initial root curling and hypocotyl elongation respectively.



Figure 2. Left: root development of lot 10 with no treatment. Middle: Root development of Ethrel treated seed from lot 2. Right: Hypocotyl elongation from GA3 blotter moistening agent treatment in lot 8.

Based on the findings of this study, it is not clear whether the Ethrel must be used only as a presoak method at 0.0145% as inferred in section 6.9h.

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## **Cool-Season Grasses Superworkshop**

By Quinn Gillespie, RST, photo credits: Angie Smith, Sharon Davidson, Quinn Gillespie

The Cool Season Grasses Super Workshop was held April 25 - 28, 2023. This workshop was a collaboration between the Northwest Seed Analysts group and the Oregon Seed Association (OSA), held in the new Agricultural Studies building at Chemeketa Community College. The workshop was very well attended with 35 people from seed companies, regulatory agencies, and testing labs all over the country coming together to learn about the specific challenges facing those testing cool season grasses. Attendees using the workshop for continuing education points were awarded 4.5 points after submitting their points to the Continuing Education Committee chairs.



Identifying specific grass seed species

Attendees received a binder with presentations and samples of over 20 species of grasses and common contaminants in grass seed crops. OSA handled the registration for the meeting, ODA was able to use grant funds to pay for the printing for the handouts and Jane Penrose from Agri Seed Testing assembled the binders and collected the seed



Touring Oregon Seed Cleaning

samples to be included.

"This is something I've wanted to do for a long time," Sharon Davidson said, referring to having a workshop that focuses on one species, or a small group of species and addresses every testing aspect of those species. The primary species of focus were tall fescue, ryegrass, Kentucky bluegrass, and orchardgrass. Specific units were taught on identification, tetrazolium staining and reading, fluorescence testing, classification of Undesirable Grass Seed (UGS) species, dividing and qualifying dividers, germination, and ploidy testing in grasses. Grow out samples of each crop were supplied by Agri Seed Testing for attendees to see the differences between cool season grass crops at maturity.

The workshop concluded with a tour of local agriculture in the Willamette valley, featuring Oregon Seed Cleaning, Red Barn Hemp, Wooden Shoe Tulip Farm, and Pure Seed Testing Lab and Research Farm. The weather cooperated uncharacteristically beautifully for spring in Oregon with clear skies and attendees were treated to a view of hot air balloons over the Wooden Shoe Tulip Farm during the tour. The workshop was sponsored by Hoffman Manufacturing, Chemeketa Community College, Oregon Department of Agriculture, Oregon Seed Association, Mountain View Seeds, Henricks Seed Lab, Saddle Butte Ag, Smith Seed Services, Barenbrug, Turf Tech Inc., and Northwest Seed Testing Inc. Beyond official sponsors, many other local companies and analysts donated time, materials, and equipment to make this workshop a great success.

There are plans to continue this type of super workshop in the future. Any residual funding from this year's registrations will be put toward supplies and speakers for future workshops.



Hot air balloons over the tulip farm



2023 Cool Season Grasses Workshop Attendees outside Chemeketa's new Agricultural Science Building



## Genetic Technology Superworkshop

By Zach Duray, Genetic Technology Committee

Kalyn Brix conducted the genetic technology workshop at the 2023 AOSA/SCST annual meeting in Saskatoon, Saskatchewan. The workshop explored adventitious presence testing strategies and methodologies, and demonstrated different tools available to technologists for planning an effective test that will produce informative results.

The fundamentals strengths and weaknesses of protein and DNA-based testing were covered. The participants discussed the nuances and effects of particle size in ground seed samples and the potential implications in testing. Several online resources were shown and demonstrated for finding information on GM events, including the International Service for the Acquisition of Agri-biotech Applications, the Biosafety Clearing-House, and the European Union Reference Laboratory for Genetically Modified Food and Feed. Participants took an in-depth look at SeedCalc in planning an adventitious presence test. Participants evaluated different testing strategies using SeedCalc, manipulating seed pool sizes and number of pools to produce different confidence and risk levels. A main takeaway from the demonstration was to consider the level of expected contamination in a sample before running a test. A test designed to detect trace levels of contamination may not be as informative when detecting higher concentrations of contamination. An analyst must consider whether a qualitative, quantitative, or semi-quantitative method is ideal for the testing goal. There are several factors to consider in a testing strategy: the number of seeds and pools to test to produce an acceptable level of risk, the cost, feasibility, turn-around time of performing the test and which traits to test for. The balance of all these factors is not standardized since every testing lab is different, but SeedCalc can be a useful tool in defining and quantifying that balance.

The last portion of the workshop was a practical exercise in modifying pool sizes to observe the effects of particle size on the estimated level of contamination in a semi-quantitative test. Participants were provided a supply of ball bearings with an unknown number of off-colored bearings representing contaminants. Ball bearings were counted randomly into pre-determined pool sizes. Once the pools were divided out, each pool with an off-type bearing was counted. The resulting number of positive pools was compared to Seed Calc's estimated contamination within the sample.

We thank Kalyn for a wonderful job organizing this year's genetic technology workshop!



## 2023 Annual Meeting in Review

By Quinn Gillespie

The AOSA-SCST Annual Meeting took place in Saskatoon, SK Canada from June 10 – June 16. There were three days of workshops, including a workshop on Statistics and Method Validation, Purity Analysis, and Germination testing. The meeting was hosted at the Delta Hotel Downtown by Mariott and name tags were sponsored by Seeds Canada. The meeting space was large and well appointed and provided a large trade show space where attendees could connect between committee meetings. There were also a large number of attendees at their very first AOSA-SCST meeting. Some had been in seed testing for several years, some just a few weeks. It's always good to see new faces join our group!

In addition to committee meetings and workshops, several research projects and posters were presented during the reception held at the trade show area.

The meeting also included a banquet held at Champetre County – Saskatoon Wild West Country Ranch in St. Denis. The buses were greeted on-site and escorted in by ranchers on horseback. Despite some rainy weather, attendees enjoyed exploring the grounds and getting up close and personal with some of the residents. At the banquet, a special service award was presented to Janine Maruschak for her dedication to AOSA-SCST as she moves toward retirement.

Due to a lower-than-expected number of SCST attendees, SCST did not have a quorum at the Business meeting, and business meeting items have since been presented to the membership and a final vote approving all business meeting items has taken place.

After the business meetings, analysts had the option to go on a tour of Saskatoon's seed and plant breeding industry, including a tour of the CFIA labs and a chance to see some of the advanced technologies presented at the meeting in action. On the flip side, analysts were also treated to the chance to see some of the very oldest seed samples in CFIA's collection. The tour also included a visit to the University of Saskatchewan Crop Development Centre and the Agriculture and Agri-Food Plant Gene Resources of Canada and Oilseed Breeding facilities. Many thanks go out to everyone who worked to make this meeting happen and especially to the Seeds Canada and CFIA participants for sharing the ins and outs of the Canadian seed industry with everyone in attendance at the meeting.



## 2023 Annual Meeting Photos

Photos by Brent Reschly & Quinn Gillespie



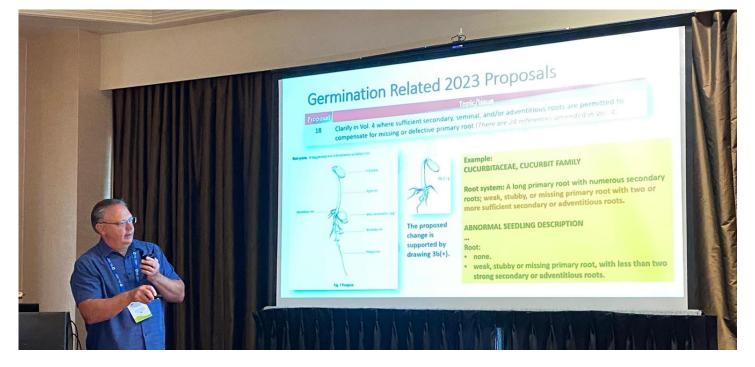
### Workshops & Committee Meetings



Dr. Lei Ren demonstrating new riffle dividers at the purity workshop (left).

David Johnston details some of the rule proposals to be voted on concerning germination. (Below)

Workshops were well attended and very informative for those who signed up. Committee meetings were all very lively and full of discussion regarding projects for the upcoming year and the rule proposals to be voted on for 2023.



### Advanced Technology Forum



Moses Palmer presenting on PCR testing of Palmer amaranth



Melissa Philips presenting on the implementation process for new technologies in the lab.

### **Champetre County Banquet and Outing**



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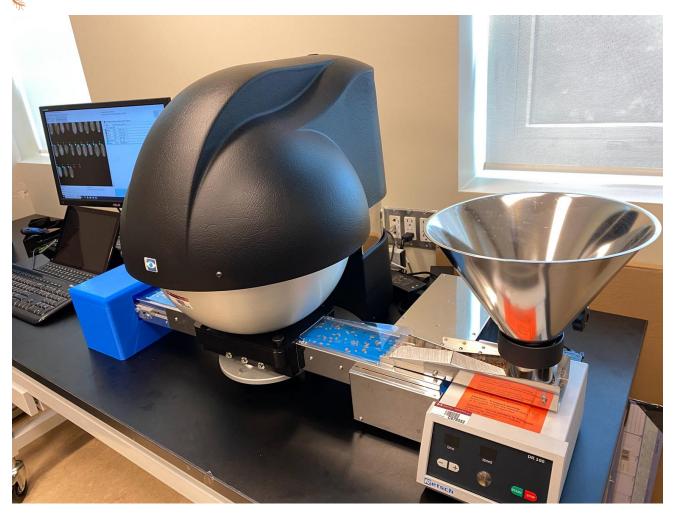


### Touring Saskatchewan's Seed Industry



Those who chose to go on the tour were able to see Janine Maruschak and Leanne Duncan in (top) and Jennifer Neudorf in their natural habitat at the CFIA laboratory.

The tour began with an overview of the virtual exam given to Canadian Seed analysts. The CFIA Herbarium has its own room with samples going back over 100 years. As expected, the herbarium room was also the hardest one to shoo analysts out of.





The CFIA lab set up a demonstration of the Videometer technology (top). The sphere is as close as possible to perfect, with an almost perfect white interior to ensure images are as standard as possible.

Unlike the US, Canadian varietal development takes place at government crop development centres, which partner with private companies. The photo (left) represents a sampling of the crops produced and developed by the Crop Development Centre in Saskatchewan. The primary focus crops in Saskatchewan are wheat and barley, and the lab includes several ovens to test the bake-ability of newly developed wheat varieties.

Analyzeseeds.com

## Method Validation & Statistics Workshop

By Quinn Gillespie, RST

AOSA/SCST and Seeds Canada, represented by Brent Reschly, RST and Dr. Ruojing Wang, hosted a workshop on statistical evaluation and method validation on June 11th, 2023. The workshop was led by the ISTA Statistical Committee Chair, Kirk Remund. Remund began the workshop with an overview of the ISTA Method Validation process and by having the workshop attendees come up with ideas of what method validation should be trying to prove, and how statistical analysis can provide quantifiable proof that a new method is effective, cost efficient, consistent, and objective.

The most hands-on portion of the workshop was in dealing with the actual experimental and statistical data used for the method validation for the newest ISTA Rule proposal regarding Salvia sp. Germination methods. Workshop

attendees were able to try out the Box Plot Excel macros developed by the ISTA Statistics Committee as a useful way to visualize data and identify outliers. If the outliers are a function of laboratory variation, they can be excluded from the dataset, but if the outliers are a function of differing methods under study they should be included in the dataset as indicative that the method may produce non-Uniform results. There was some excellent discussion of how outliers can still provide valuable information when it comes to examining seed from a single lot, and can help pinpoint nonuniform storage or shipping conditions.

Attendees were also asked to examine different spreads of data for trueness and precision. Trueness being measured by whether or not the data is biased, and precision is measured by standard



Kirk Remund and workshop attendees taking a hands-on approach to statistical analysis.

deviation. Data which is true, but not precise may require more study and data points to hone in on quantifiable trueness. Data which is precise but off-center may represent a different truth than expected, or a need for recalibration of the results or method. Both types of data were demonstrated to be very useful when proposing new methods and evaluating them for reproducibility.

One of the projects of the ISTA Statistical Committee has been to re-evaluate the work done by Miles with regards to reproducibility and they have found that the original studies conducted in 1953-1954 and 1955-1959 used to determine the reproducibility determination factor still hold up today. This is an excellent example of how solid experimental design and statistical analysis can produce results that are reproducible and accurate decades later.

Many thanks go out to Kirk Remund, and his co-chair Dr. Jean Louis Laffont for the informative presentations and development of useful tools for statistical analysis when analyzing methods. The Box Plot Macro worksheet and other tools developed for statistical analysis can be found on the <u>ISTA Statistical Committee</u> website.



### **Germination Workshop**

#### Brandi Taylor

The Germination Workshop was held on June 10<sup>th</sup> at the Delta Hotel in Saskatoon, SK prior to the Annual Meeting. The initial focus of the workshop was on uniformity in seedling evaluation and the ways that individuals and laboratories can demonstrate uniformity in seedling evaluation. Most labs have processes in place to ensure uniform seedling evaluation between analysts in a single lab; but it can be more difficult to demonstrate uniformity and reproducibility between separate labs. During the workshop it was emphasized that a lack of uniformity between labs can result in stop sales, failure to meet company contracts, variation between seed production results and test results at the destination, and customers losing trust in lab results.

When dealing with natural materials, there is always some innate variability in biological materials. Poor sampling procedures and lot heterogeneity increase the likelihood of variability in the final test results. In all cases, the test results are only as good as the sample received. Reproducible germination results start with good sampling procedures. With a good, representative sample labs can control for further variation by using validated testing methods, maintaining equipment and necessary calibrations, and ensuring and documenting analysts are thoroughly trained for the seed type being evaluated.

Part of that necessary analyst training is an understanding of the principles of germination testing. In a seed lab a germination test is designed to assess the potential of a seed to produce a normal plant under favorable conditions. In Volume 1 of the AOSA Rules, Table 6A lists the methods that have been approved for germination testing of hundreds of species. However, the methods also frequently include multiple options for substratum, temperature, lighting, and dormancy breaking procedures. This gives analysts flexibility in adopting a method which suits their capabilities as a lab, but also introduces a source for significant variation between laboratories. Two important questions posed by the workshop, and part of the ongoing work of the research conducted by seed analysts, is "Are we really using the best method to achieve the highest potential of the seed?" and "Which method produces the least amount of variability between labs?"

Variability between labs can be assessed by applying tolerances, as detailed in Chapter 14 of Volume 1 of the AOSA Rules. The tolerance tables are designed to account for unavoidable random sampling variations between germination repetitions and tests conducted on the same or different samples from the same seed lot. Any human component of intolerant results can be reduced by ensuring that analysts are trained to evaluate seedlings both correctly and consistently, and by validating the methods used in seed testing for reproducibility between labs.

The second portion of the workshop focused on seedling evaluation according to the AOSA Rules for Testing Seeds. Volume 5 of the AOSA Rules includes many drawings, descriptions, and notes to help analysts evaluate seedlings correctly with new rule proposals focused on adding additional drawings and photographs to aid analysts in their evaluations. A rule proposal was introduced at the 2023 Annual Meeting to add photographs to aid in the evaluation of lettuce seedlings which can be especially difficult to analyze due to the subtlety of physiological necrosis when present.

With an eye to continued improvement the Germination Committee is also developing tools to improve analyst training and reduce variability between labs. During the workshop attendees saw a demonstration of the Seed Image Database, which is the result of many hours of work and surveys of analysts all over the country to produce a reference library of evaluated seedlings. This database will be a large step forward in reducing some of the subjective nature of seedling evaluation. Attendees also participated in three exercises in evaluating specific abnormalities, such as necrosis in lettuce seedlings. This exercise encouraged analysts to evaluate seedlings first by percent of healthy tissue, then by percent of damaged tissue to see how that changed their perception of each seedling.



For seed testing labs and our customers, both internal and external, uniformity is a vital concern. Seed labs should always be doing everything within their control to mitigate variables within the lab and be open to trying other methods to ensure the testing methods used achieve the highest potential of the seed. While biological variation is inherent in germination testing , new tools are being developed to help laboratories and analysts provide consistent results across the industry.



### Advanced Technology Forum

#### By Quinn Gillespie

The Advanced Technology forum was held on June 12, 2013. This was forum was organized by Ruojing Wang, Brent Reschly the chairs of the new Advanced Technology Committee, and Krista Erickson from Seeds Canada. Four different technology providers gave presentations of new technologies which may be of interest and benefit to seed testing laboratories. These presentations were followed by presentations from members detailing the current advanced technologies already in use by their labs with advice about implementation and how to choose which new technologies make the most sense for the individual lab.

The technology presenters started with Anitje Wolff, from Phenolytics, who attended virtually. Phenolytics has developed phenoCheck, which produces a 3D scan of the interior of the seed, showing the internal structures. This can be used to examine the embryos present inside pelleted seed and to identify underdeveloped seed. Phenolytics has also developed a system called phenoTest, which is an automatic germination program, which controls moisture, and produces a reconstructed 3D image of seedlings in grayscale, which can then be color coded to identify and measure the cotyledon, hypocotyl, and root tissue. This non-destructive process also allows the seedling to be measured over time and can currently be applied to 60 different species. With regards to uniformity, the ability to quantify the amount of cotyledon, hypocotyl, and root tissue could be very useful in laboratory testing in the future.

Taylor Scott presented on the capabilities developed by Skyway Analytics and Videometer. Skyway Analytics is an agriculture-focused imaging company which uses multispectral imaging to analyze many different aspects of seed purity, and seed appearance. Some of the technologies developed are specifically geared toward phenotyping, ploidy, and categorizing off-types, as well as analyzing the amount of coating or pelleting adhered to seeds. The Videometer compares 19 different wavelengths of light and uses machine learning software to measure the physical features and identify different seeds. The laboratory at

CFIA uses Videometer technology and Ruojing Wang demonstrated the capabilities of the imaging software on a sample of barley contaminated with flax seeds. The machine was able to accurately photograph and count the number of contaminants present.

Jill Gagnon from Tagarno presented information about the new Tagarno digital microscopes, and had a table set up during the trade show for analysts to try out some of the Tagarno scopes themselves. The base magnification range for most of the Tagarno scopes starts at 1.7x and can be increased to 53x. Tagarno scopes also have the ability to interface with web-conferencing applications to allow analysts



Jill Gagnon demonstrating the capabilities of the Tagarno digital microscopes at the trade show.

all over the globe to look at exactly the same seeds. Multiple AOSA and SCST labs are already using these scopes and can appreciate the ability to capture crisp, close-up images of seeds for use in identification.

Lee West from hiphen shared some of the possible solutions for harmonizing seed testing assays, using full-stack image analytics. Hiphen works primarily to develop applications for plant phenotyping and evaluation of trial quality, biomass proxy, plant stress, and harvest quality on crops in the field. For seed testing, seed analysts would provide the validation for future image analysis and serve as subject matter experts.

Further presentations in the applications of advanced technology were presented by representatives from SCST and Seeds Canada member labs.



Lee West presenting some germination solutions from hiphen.

Melissa Philips presented some of the questions that labs need to ask themselves when implementing new technology. Some of the key factors to consider are what problem is being solved with technology? Is the implementation going to cause its own problems? How do you ensure that the new technology is still going to provide test results in accordance with the AOSA Rules? Some of the benefits presented are the ability for technology to perform repetitive tasks, freeing up analysts to perform human-critical tasks. Technology can remove subjectivity, and when it makes a mistake- it makes the same mistake over and over, not new ones.

Moses Palmer from 2020 Labs presented some of the research done to use PCR analysis to identify Palmer amaranth seeds, a contaminant which has been an important topic of discussion over the past five years as more and more states classify Palmer amaranth as noxious. He detailed the problems of Palmer amaranth, primarily that a single plant can produce millions of seeds which then compete with the crop kind for water, space, and nutrients, and can lead to significant yield loss. It can also be difficult to distinguish A. palmeri from non-noxious types visually, necessitating the need for an additional test to positively identify the seeds. The PCR test conducted by 2020 Labs can be done in pools of 1-25 seeds and can be used for tissue samples to precisely identify A. palmeri. Outside of the presentation I was able to touch base with Moses, and he said there is also some preliminary work being done on A. blitoides, which can be a phytosanitary concern when sending seed to some countries.

Noureddine Mehaihi from CFIA presented on the applications of AI solutions and predictive modeling, and how analysts can work with multispectral imaging to develop seed identification solutions. He emphasized the importance of collaboration when developing deep neural nets and imaging protocols. His

focus is primarily on using open-source models for public use, to cast the widest net possible for collecting images, data, and validation of methods and AI machine learning.

Monica Garcia from Corteva was able to share the results of implementing automatic planting technology for germination testing in her lab. Some of the drawbacks were a larger upfront cost, specialized knowledge to set the planter, a large footprint, and the necessity of contacting an engineer to make changes and repairs. The benefits are the reduced repetitive tasks for human laborers, increased efficiency, speed, and standardization. The planter arranges 50 seeds at a time, which are then counted via camera image and machine validated, and a barcode is generated. Samples are mostly planted between paper. ANOVA analysis was used to prove that the planter results are reproducible and repeatable.

The forum was concluded with a panel discussion in which the both virtual and in person attendees could ask questions about the implementation of new technologies and specific issues that have come up. One of the primary concerns was cost, with Steve Jones asking what some of the base rates for advanced technology can be, with an eye to the cost for labs in less-developed countries which may not have consistent internet access or the footprint to implement large-scale automation. It was also mentioned that newer technologies attract new talent, and an upfront cost can be used to offset additional labor costs down the line. Overall, the advanced technology forum provided many interesting concepts and potential applications for machine learning and automation regarding seed testing for analysts and laboratories to explore in the future.

Many thanks to Ruojing, Brent, and Krista for organizing these presentations.



The Advanced Technologies Forum was very well attended both virtually and in person.



#### Lauren Mezo, CVT

Lauren joined the MICA team in July of 2020. She fills a dual role, assisting with field inspections during the summer months and works in the lab the remainder of the year.

Lauren comes from a cash crop farm in Galesburg, Michigan where she gained her appreciation for agriculture. She earned her Bachelors Degree from Michigan State University in Agribusiness Management.

Out of the MICA office, Lauren enjoys exploring new hiking trails, traveling, and spending time with her family and better half, Mitch.

#### Tammy Stark, CVT

Tammy joined the Hubner Industries Quality Lab in 2009 working in germination. In 2019 she was introduced to Michael Stahr who shared information with her about SCST. This is what started her involvement with the SCST as she became an Associate Member and started learning about being more involved in the seed testing industry. She attended the AOSA/ SCST Annual meeting in 2022 and enjoyed meeting more people within the industry and gaining more insight into the seed testing community.

She is also actively involved on the Communications Committee for ASTA.

Tammy grew up on a small family farm in Arkansas,

but currently resides in a small Indiana town. She has three children and one grandson, all of whom keep her very busy. Her children and friends would say she enjoys attending sporting events, liberal arts events, traveling and especially visiting other labs when she is away from work.





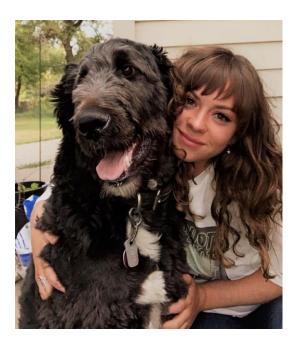
#### Zach Duray, CGT

I grew up in the Northwest suburbs of Chicago and decided to pursue a career in agriculture with a focus on plant genetics. I have my Bachelor's Degree in Crop Science with a concentration in Plant Biotechnology and Molecular Biology, and my Master's Degree in Plant Biotechnology under the Professional Science Master's program, both from the University of Illinois at Urbana-Champaign.

My working background is primarily in plant pathology research; I served as a technician with the USDA Agriculture Research Service and the University of Illinois. While with the USDA, I worked with the Wheat, Sorghum, and Forage Research Unit in Lincoln,

Nebraska, performing disease resistance bioassays on transgenic wheat. The goal of the experiment was to determine if manipulated regulation of the monolignol biosynthesis pathway played a role in Fusarium head blight resistance in wheat. At the University of Illinois, I was the Visiting Research Specialist for the Applied Field Crops Pathology Research Lab in the Department of Crop Sciences. In addition to managing the lab space, my primary focus was on a tar spot genotyping and mapping project. The objective of this project was to map the distribution and deviation of fungal barcoding genes in various isolates of *Phyllachora maydis* in the form of phylogenetic trees to better understand how tar spot differed genetically from its origin in Mexico, to new sightings in the Midwest. In 2020, I joined the Illinois Crop Improvement Association as the Field Services Director. I manage the field inspection program for seed certification in Illinois and oversee the trait testing lab and greenhouse. At Illinois Crop, my bioassay experience and genetic knowledge are frequently utilized when performing herbicide bioassays and ELISAs to test for trait purity.

In my free time, I enjoy all forms of science-fiction media, mostly through audiobooks and movies. Currently, I am listening to the sixth book in Frank Herbert's Dune series. I like to stay active by going for bike rides and walks, and am passionate about martial arts. I've actively trained in taekwondo for the last 13 years, and currently hold a second degree black belt. I started attending classes this year to learn Brazilian jujitsu. I'm still active on the University of Illinois campus, volunteering as a taekwondo instructor, and invited occasionally by the graduate college to talk to students about professional development and how to prepare for careers post-graduation. I usually take my coffee black; I enjoy a nice dark roast.



#### Mackenzie Mattern, CGT

I am an experienced Genetic Technologist at SoDak Labs with a proven track record in the field of hard sciences. Throughout my academic journey at South Dakota State University, I actively engaged in diverse organizations. Notably, I assumed the role of President for the Horticulture and Urban Agriculture club for a significant portion of my four-year tenure. Moreover, I was privileged to be a member of esteemed societies, contributing in capacities ranging from general membership to treasury positions.

During this period, I gained hands-on experience as a biological technician's assistant and intern, participating in a range of projects spanning from investigating insect pesticide resistance to assessing the neonicotinoid impact on lady beetle reproductive development. In recognition of my contributions, I was awarded the Griffith Undergraduate Research grant during my junior year. This grant supported my exploration of native pollinators and their implications on conventional sunflower yield. The research outcomes provided opportunities for me to present at various national conferences, fostering my presentation skills and facilitating my initial foray into academic publishing.

Subsequently, I translated my expertise in native pollinators into a one-year master's program, building upon the data amassed during my undergraduate studies. Upon graduation, I transitioned into the seed testing industry, gaining proficiency in ELISA trait testing encompassing both quantitative and qualitative aspects. This foundation led to my progression into an analytical scientist position, where I undertook responsibility for a diverse array of assays, including reduction extraction for amino acid analysis and quantification.

Re-engaging with biology at SoDak Labs, my current role involves spearheading, coordinating, and overseeing the execution of corn isozyme electrophoresis and a spectrum of seed health assays. I possess comprehensive training in ELISA trait-purity, lateral flow strip methods, and PCR adventitious presence testing.

Beyond my professional pursuits, I find solace in literature, relish the company of my canine companion Tango during leisurely park strolls, and cherish moments spent enjoying a refreshing beverage with friends.

#### Lauren Shearer

#### Lauren Shearer

Graduated South Dakota State University with bachelor's degree in animal science in 2013. Worked at SDSU's Beef Reproduction Research Laboratory, then at Alltech's In Vitro Fermentation Laboratory, and then completed a master's degree in Dairy Production, focusing on ruminant nutrition in 2018. That same year I started at SoDak Labs as a PCR technologist working to set up GMO screen methods along with their genetic purity with isozymes. Basically, for the last ten years I have been a "Lab Rat" but have enjoyed learning a variety of lab techniques and methods. When I'm not in the lab, I enjoy baking, crafting with the cricut, or relaxing with a good book but most often than not I'm chasing after my kids on their adventures. Favorite sayings are "A great leader is a self aware leader" and "Continual improvement is better than delayed perfection."

### AOSA & SCST Award Recipients



#### Jane Penrose, RST, SCST Meritorious Service Award

#### Profile submitted by Sharon Davidson

It was in the spring of 1986 that Rod Bowdish phoned me and asked if I would give his daughter a job. Jane Bowdish Penrose was graduating from Western Oregon University with a BS in Biology. Jane started working at Agri Seed Testing, Inc in June of 1986. She immediately showed an interest in seeds and the ability to identify each one. Jane obtained her RST just two years later, in 1988.

Since then, Jane has trained hundreds of people in seed testing. Every year, past employees stop by to thank her for the training they received while they

worked with her during their summer breaks. She instills the same work ethic in them that she was raised with and it helps when these folks go out in the real world. They learn life skills as well as seed purity testing skills with Jane as their supervisor.

She works with many of the seed conditioners in Oregon as well. Teaching them how to identify contaminants, taking photos to show contaminant size compared to the crop for setting cleaning machines, and giving them advice on how to pull a certain problem seed out of the crop.

Jane has been an active member of SCST serving on the Executive Board in 2006 - 2009, also serving on several committees, proposing rule changes, and providing input for the betterment of seed testing as a whole.

Two years ago, Jane was instrumental in helping our local community college start a new curriculum in agriculture. She sat on the committee that created what is now a well-rounded two-year degree for people in agriculture with exposure to seed testing.

In April 2023, Oregon hosted a Mega Cool Season Grass Workshop. There were 35 people in attendance from all corners of North America. Jane was instrumental in teaching several of the topics as well as putting together the take home binder with the presentations, worksheets, and quizzes. She also prepared 40 seeds from the grass family into coin holders for each participant to take home for their identification purposes, many from the list for exam takers to know.

In the lab, Jane has implemented the QA program for Agri Seed. She understands the concept as a whole and can see ways to enhance the credibility of our results with monitoring and maintenance. She has a full working knowledge of all rule sets and can apply each as needed. Currently, she is meeting with the purity department weekly, teaching in detail each Pure Seed Definition with examples and graphics.

Jane is a true leader. Other supervisors within Agri Seed Testing and from other labs respect and look to Jane for help when they have a difficult situation. She has truly devoted her life to seeds and her enthusiasm about them comes through in all she does.

### AOSA & SCST Award Recipients



## Janine Maruschak, Special award for service to AOSA and SCST

Profile submitted by Brent Reschly

Janine grew up in Saskatchewan on a farm and was surrounded by agriculture, nature and plants, and has always had a great appreciation for that. Janine studied at the University of Saskatchewan. She celebrated 33 years of service with the federal government this year and plans to retire at the end of 2023. Janine has been the Head of the Seed Science and Technology Section at the Canadian Food Inspection Agency's (CFIA) Saskatoon laboratory since 1999 and currently manages a team of 27 seed testing colleagues. Janine has been a

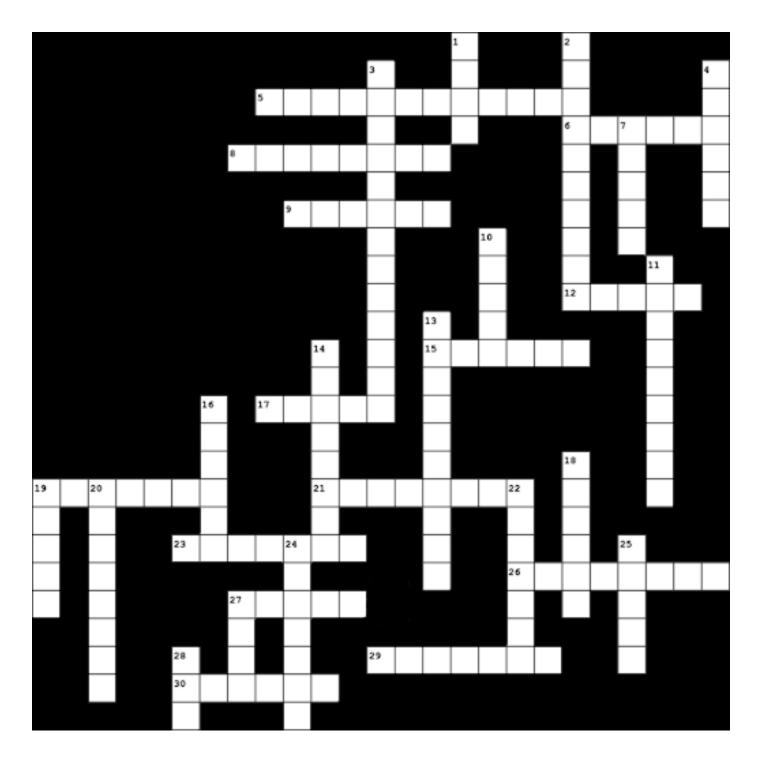
participant at the AOSA meetings since 2000, has served on the Rules Committee, the Referee Committee and has been an Executive Board member since 2006 and Secretary Treasurer since 2011.

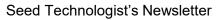
Janine continues to be actively involved in AOSA, having hosted the AOSA meeting in Saskatoon in 2005 and 2023. Janine is also an internal and external auditor overseeing the CFIA's accredited seed laboratory program and is actively involved in the Canadian seed sector serving on the CSAAC board, has attended OECD, ISTA meetings and has a great deal of experience in seed certification and grain testing systems. Janine sees it as a privilege to be able to work with many staff of different expertise, who come from different back-grounds with different experience and have skill sets to do the work that SSTS does on behalf of Canadians and the seed sector. Janine rocks! She will be sorely missed and both the AOSA and SCST wish her all the best in retirement.



### Study Guide: PSUs and Seed Structure

By Quinn Gillespie







#### Across

- 5. This super-chaffy grass has multiple PSU classifications and special uniform blowing procedures.
- 6. A hardened area at the base of many grass florets.
- 8. Free from hairs, smooth
- **9.** A structure of bristles, hairs, scales, or awns at the tip of some Asteraceae seeds.
- 12. The juncture between 6 across and 20 down. U shaped or V shaped, can be helpful when identifying wheatgrasses
- **15.** Seeds which may be difficult to separate due to their structure or texture. The superlative version might wear a cape.
- **17.** The upper 'inside' bract enclosing the grass caryopsis
- 19. With bristly hairs
- 21. Fruit wall, often persistent in seeds.
- **23.** The stalk of a floret in an inflorescence or of a grass spikelet
- **26.** Growth around the hilum, common to euphorbiaceae
- 27. A leaf-like structure at the base of a grass floret
- **29.** The most common way to find wild garlic as a contaminant.
- **30.** Even if one of these is living inside a pea seed, it's a pure seed if unbroken and has a portion of seed coat attached.

#### Down

- 1. This is not part of the pure seed for 10 down, but can be left on silky oak
- 2. The free caryopsis of \_\_\_\_\_ is inert if less than 2mm in length
- 3. This genus of daisy comes in two different shapes.
- 4. With stiff rigid hairs
- 7. The lower 'outside' bract which encloses the grass caryopsis
- 10. Genus for Eastern and Western hemlock
- 11. This 'umbilical' cord may leave behind remnants on the seed.
- **13.** Seeds with a split nature
- **14.** This must extend past the tip of the floret in a grass seed to count.
- **16.** 1 single indehiscent fruit
- 18. The little whirligigs dropped by maple trees
- **19.** If seeds had a belly button this would be it. Shape, position, and size can be used to help identify many species.
- 20. The main axis of a grass or cyperaceae spikelet.
- **22.** This family must be decoated before conducting a purity examination
- **24.** an insect that damages seeds, leaving them puffy, soft, or dry and crumbly.
- **25.** A field been seed with cotyledons split apart and not held together by the seed coat.
- 27. Caryopsis length in grasses is typically measured from the \_\_\_\_\_ of 20 down.
- **28.** A stiff bristle growing from the tip of the lemma in some grasses.



### Genetic Technology: Grab Bag Pop Quiz

Written and submitted by the Genetics Technology Committee

1.) What is the term for introducing a DNA construct into plant cells?

2.) Name three main factors that effect separation in gel electrophoresis.

3.) You have 400 seeds in a herbicide bioassay. 10 are abnormal. 10 are dead. 10 are susceptible. What is your percent tolerant seedlings?

4.) In which type of immunoassay does the test sample flow along a solid substrate via capillary action?

5.) Which species of bacteria is a source of thermostable DNA polymerase making PCR possible at high temperatures?

6.) Name the two gel parts of a PAGE gel.

7.) What type (not brand) of herbicide is glyphosate?

8.) What is the term for RNA synthesis in the central dogma?

9.) What is the term for the lowest amount of analyte in a sample that can be detected with suitable confidence?

10.) In DNA, guanine pairs with what?

### Lost Resources

#### Reginald Denny Hall Jr.



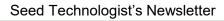
Denny Hall passed away after a 2 year battle with cancer. He was at his home in Powell Wyoming with his wife Jackie and sister Patty Mayfield by his side.

He was born on December 1, 1953 to Donna and Denny Hall in Morgantown,West Virginia. He graduated from Morgantown high school in 1971. He was active in FFA and attended the National Convention in Kansas City as part of a dairy judging team. He attended Glenville College in West Virginia, receiving an associate degree. After working as an extension agent he returned to the University of West Virginia. He received a degree in Animal Science. He worked 2 years as a Conservation Officer before attending Montana State University where he received a Masters in Agronomy in 1988.

After graduation Denny worked at the Plant Material Center in Bridger, Montana. He then was employed by the University of Wyoming as the Seed Certification Director. He encouraged the University to move the service from Sheridan to Powell where the majority of the seed crops were grown. In 2000 he returned to Bozman, Montana to train as a Seed Analyst. He returned to the new Seed Lab in Powell as assistant analyst eventually becoming the Director.

He worked hard to develop good working relations with legislators, growers and the Department of Agriculture, all of whom were instrumental in bringing the State Seed Lab to Powell. During his years at the lab, he served on the AOSA RST Exam Committee Review Board and the Native Seed Committee. He was especially proud of this group as they developed testing standards for native species that insured uniform procedures nationwide. During his tenure he developed a reputation as the "go to lab" when testing native species for the BLM and Reclamation companies.

Denny was an avid fisherman and gardener. He loved to observe wildlife with his spotting scope. At one point in his life he took up photography and briefly played electric bass in a band. He continued to love music especially bluegrass and traditional western performers. He grew up in the Goshen Baptist Church in Morgantown and attended the First United Methodist Church in Powell. He served as an usher for services and funerals as well serving as a trustee and head of the council.



Denny is survived by his wife Jackie of 35 years, as well as his sister Patty Mayfield (Gary), nieces Crystal Frankenberry (Bryan), Wendy Barker, Stacy Logan, Heather Faxon (Paul), great-nephews including Jake and Zeke Frankenberry, Noah Faxon, Mitch Mayfield, Greg and Trey Randolph and great-niece Raven Moore (Clayton).

Denny is proceeded in death by his parents, Donna and Denny Hall, his brother Butch, nephew Jeff Mayfield and nephew-in-law Vince Barker.

A service is planned for 10am on July 19, 2023 at the First United Methodist Church in Powell. Memorials may be sent to the church at PO Box 778, Powell, Wy 82435

To send flowers to the family in memory of Reginald Denny Hall Jr., please visit our flower store.

### Lost Resources

#### Vicky Calderon



Vicky was born in Nampa, Idaho on March 3, 1945, to Andrew F. Voigt and Goldie Peterson Voigt. She was raised on a farm East of Nampa and attended country schools until they were consolidated with the Kuna schools. Upon graduating from Kuna High School in 1962, she continued on and attended Kinman Business College in Spokane, Washington. There, she graduated in 1963 with a secretary degree. In 1964 she went to work at Northrup King Seed Company in Nampa. She met and married Earl E Wright in 1964. They later divorced. She then married Richard G Caldon, December 26, 1970. Vicky trained to be an in-house seed analyst and took the Registered Seed Technologist test in 1977. She worked in Boise for Northrup King and the Idaho State Seed Lab. Then she transferred to Oregon to work for Roberts Seed Company in Tangent.

In 1995, she opened and operated her own seed lab in Halsey, Oregon until Richard passed away in 2000. The next few years she was a rover between Nampa and Richland, Washington – only to finally settle back in Nampa permanently. She is preceded in death by her parents; her husband, Richard Caldon; and her siblings (Carl, Leo, Jerry, Thelma, Helen & Frankie). She is survived by sister-in-law, Phillis Voigt of Fruitland ID; brother-in-law, Carl Drapeau of Springfield OR; several nieces and nephews; long time friends, Claretta Evans & family and Rosalind Borcher & family.

It was Vicky's wishes that no services be held.

To <u>send flowers</u> to the family or plant a tree in memory of Vicky A (Voigt) Caldon, please visit the <u>Nampa Funeral Home</u> floral store.

Obituary as published by the Nampa Funeral Home.

# AOSA SCST 2024 Annual Meeting

# Rapid City, South Dakota June 2-6, 2024

Check for updates here: <u>https://analyzeseeds.com/annual-meetings/</u>



### Host Hotel: Holiday Inn Rapid City Downtown

•\$139.00/night

- •Complimentary Self-Parking
- •Complimentary WiFi in Meeting Rooms and Guest Rooms
- •All meeting events will take place at Host Hotel

•Located next to Memorial Park and walking distance to Downtown Rapid City



### Getting to Rapid City & Things to Do

Rapid City Regional Airport

- •10 miles from the hotel
- •Major hub for multiple airlines

Once you arrive...

- •Mount Rushmore National Park: 25 miles
- •Badlands National Park: 62 miles
- •Explore downtown Rapid City: Museums, restaurants, parks, and more!
- •Visit: https://www.visitrapidcity.com/

