Seed Laboratory Report Q1 - Q3+ July 2021 – April 2022

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5-24-2022



CDFA Seed Lab Functions

- Regulatory Samples Purity, Noxious Weed, and Germination
- Seed Investigations
- Service Samples Identification, Purity, Germination, Seed Vigor
- Identifications for County Ag Commissioners (high-risk dog team interceptions), Border Inspection Stations, private labs
- Association of Official Seed Analysts (AOSA) streamline national seed analysis rules and improve uniformity in analysis
- Training new seed analysts for the future





CDFA Seed Lab-sample dividing, purity, herbarium, germination

Purity analysis

- Pure seed unit (PSU) and required working weight for each crop
- Each sample approximately 2500 seed units by weight
- Each sample is separated into pure seed, other crop seed, weed seed, and inert matter (soil, sticks, chaff, broken seeds, plant parts, fungi)
- Test these 4 components for compliance using Federal Seed Act or AOSA tolerance tables
- Our lab routinely tests over 100 different crop species









Purity Components: Pure Seed, Other Crop Seed, Weed Seed, Inert Matter

Seed Mixtures

- Regulatory Samples over 50 crop species mixtures per year
 - Examples turfgrass mixtures, grain mixtures, forage mixes, erosion control mixes (commonly 2-8 species per sample, one 17-species sample this year)
- Seeds of all species must be completely separated by hand for purity analysis and each species tested separately for germination
- Preparation time for mixtures ranges from 3 to 20 hours per sample

Noxious Weed Seed Examinations

- Conducted on all regulatory samples of agricultural crop seeds
- Each sample approximately 25,000 seed units by weight according to Federal Seed Act and AOSA Rules
- Zero tolerance for Prohibited Noxious Weed Seeds (stop-sale order)
- Restricted Noxious Weed Seeds must be within tolerance of zero per sample (maximum of 2 seeds per 25,000) or listed on the label



California Noxious Weed Seeds

- Over 180 species are listed as noxious weeds in California Code of Regulations
 - Prohibited noxious weed seeds
 - Restricted noxious weed seeds
- No tolerance for noxious weed seeds in certified seed lots

Photo: Scotch thistle, a prohibited noxious weed seed that badly degrades rangeland; D. Meyer, CDFA Seed Lab

Giant hogweed, *Heracleum mantegazzianum*, a Federal noxious weed

- Old World species
- Established in the Pacific Northwest and eastern U.S.
- "A"-rated pest in California subject to exclusion and eradication
- Hazardous to humans and livestock
- Not currently known to occur California

Photo credits: Plant, L. Mehrhoff, Univ. of Connecticut, Bugwood.org; Fruits, J. Scher, USDA APHIS, Bugwood.org





Seed Investigations

- We provide unbiased analyses to help resolve seed disputes or problems reported by the Seed Control officer
- Can help protect seed sellers
- Examples:
 - Unusually low or variable germination results
 - Poison control referrals poison hemlock plants in a vegetable garden near carrots planted from seed
 - Seed testing result poison hemlock likely present in the environment and not from the carrot seed



Photos: Plant, Ohio State Weed Lab, bugwood.org; Seeds, Steve Hurst, bugwood.org

Seed Identification at CDFA

- Service Sample Seed Identifications (39 samples in fiscal year)
- Seeds from global trade found as contaminants and unable to be identified by the private labs (92%)
- Can't sell the seed lot unless excluded species are ruled out
- Intercept samples (18% were of prohibited noxious weed seeds):
 - Seeds found by dog-team intercepts of parcels (13)
 - From vehicles at CA Border Inspection stations (25)
 - County high-risk interceptions (36)

Dust-like seeds

- Very small (0.5 mm or less), found in a variety of flowering plant families, including Orobanchaceae and other families formerly grouped into the snapdragon family
- Some non-native taxa of Orobanchaceae (e.g., Orobanche) are Federal noxious weeds parasitic on important crop plants such tomatoes and hemp
- Very expensive and difficult to control due to longterm seed bank
- Morphological identification of dust-like seeds is challenging

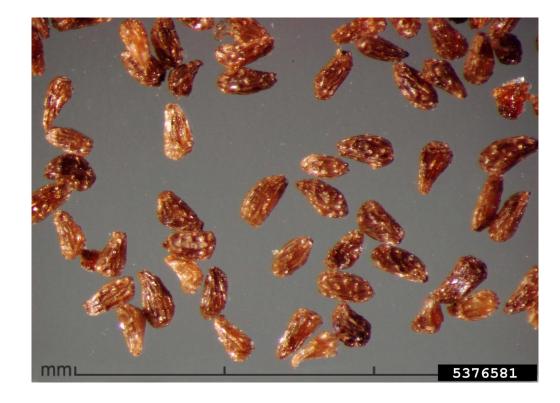


Photo: Julia Scher, USDA APHIS, bugwood.org



Seed of Calceolaria (slipper flower) found in a Brassica vegetable sample

- Seed 0.3 mm
- submitted for identification by a major seed company lab
- DNA sequence 99.7% identity match to *C. corymbosa*, a native South American wildflower.
- Plant is cultivated globally as an ornamental flower
- Seed may have lodged in dividing equipment



Photo: A. Lomas, www.flickr.com

(Photo: D. Meyer, CDFA Seed Lab)





Garlic mustard, noxious weed new to California

- Noxious weed in the northern U.S.
- Discovered in California in the last two years
 identified by the CDFA Seed Lab
- Under eradication by the U.S. Forest Service
- Develops a lasting seed bank
- Inhibits growth of crop plants
- Should be kept out of farmland and agricultural seed supply

Thanks!

 Bob Price, Senior Seed Analyst, CDFA Seed Lab

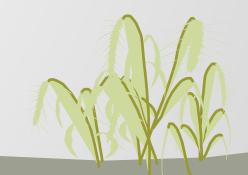
Mantisalca salmantica, daggerflower, an "A" rated pest sold in craft stores. Photo: D. Meyer, CDFA Seed Lab





Seed Lab (SL) Functions
 SL Label Compliance Testing Activities

Seed Testing: Regulatory Perspective
 Training and Methods Development: Essential Activities
 Benefits to the Seed Industry

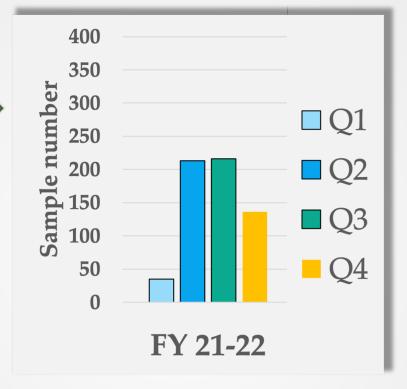


Sample number and distribution

Total (July 2021-April 2022): 528
(88% of 600 target)

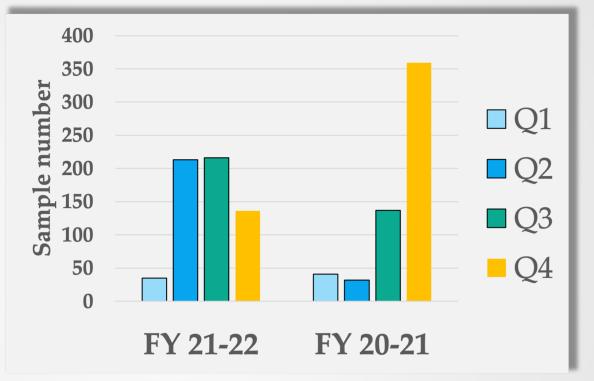


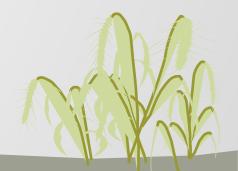
Sample number and distribution





Sample number and distribution

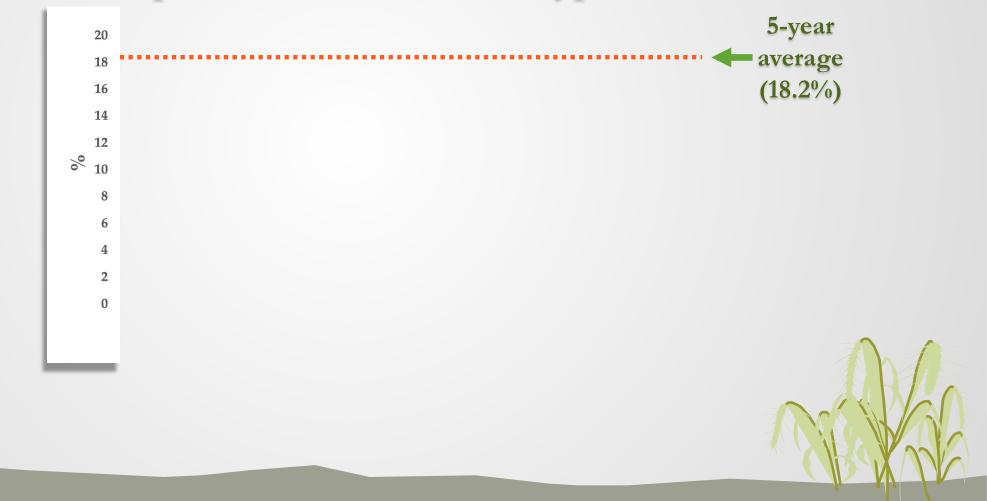


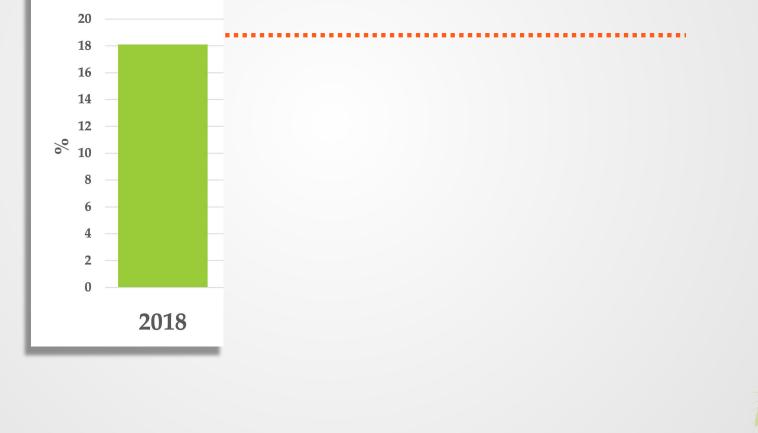


Sample number and distribution Label violations

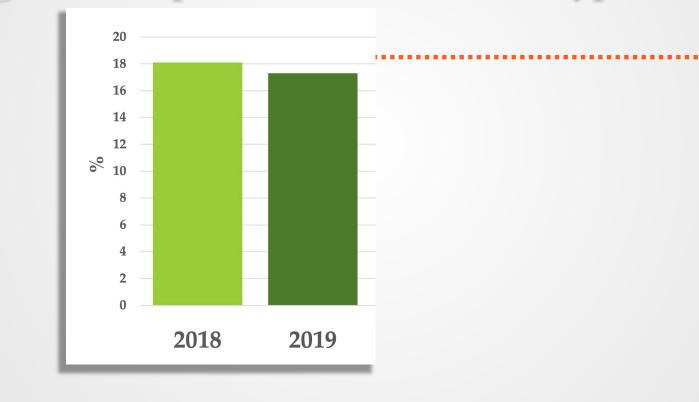
Labelling standards
 Purity
 Germination



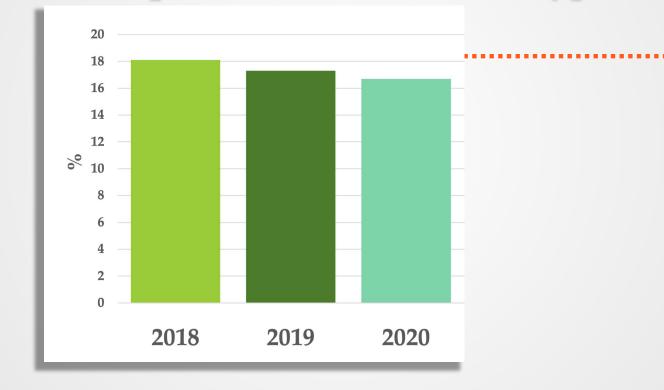




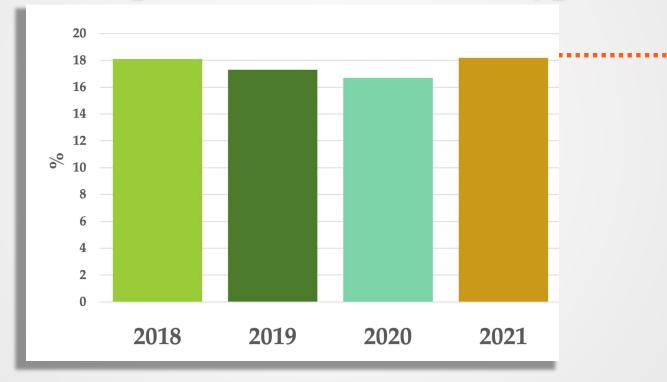




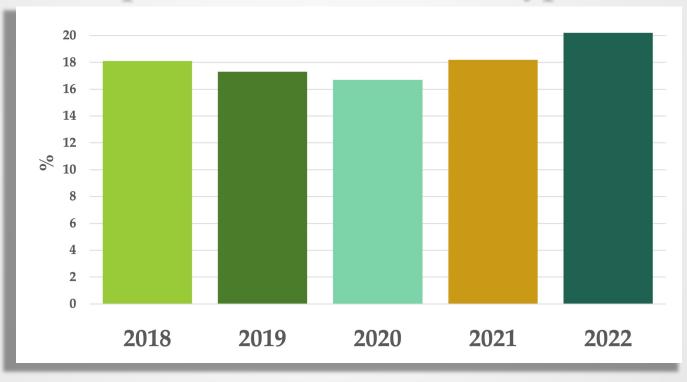


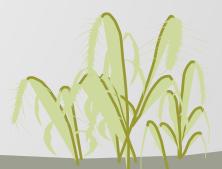






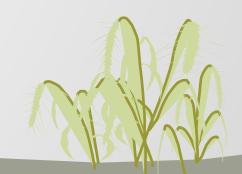








PVP notification; Seed kind; Non-registered labeler

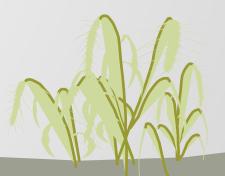


Sample number and distribution Label

violations

Purity/Germination Violations:

- Pure seed percentage(5%, n=26)
 Inert matter percentage(3%, n=14)
 Other crop seed percentage (1%, n=4)
- Germination percentage (1%; n=4)

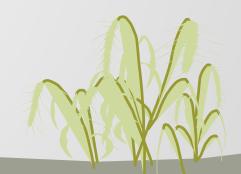




Service testing (for fee) 281 samples ✤ 171 rice for certification 26 seed IDs 19 custom tests Feed mill inspections (48) Detections/identifications Training/methods development **Research**/investigations >Other services

What is Seed Testing? Regulatory Perspective

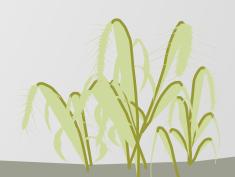
Rules: application of standard procedures to evaluate physical, physiological and other characters of seed lots.



Seed Testing

Rules (standard procedures):

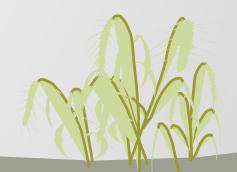
For all aspects of seed testing
 Association of Official Seed Analysts (AOSA)
 Federal Seed Act (FSA)
 International Seed Testing Association (ISTA)
 Other country-specific rules (e.g., Canadian M&P)



SOPs vs. Rules

SOPs:

Documentation, safety measures, equipment inspections, training requirements, etc.
 Testing procedures (Rules)
 must be the same across labs



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Table 2A. Weights for working samples

Pure Seed Unit #	Chaffy (C) or Super Chaffy (SC) ⁸	Kind of seed	Minimum weight for purity analysis ^b Gram s	Minimum weight for noxious weed seed or bulk examination Gram s	Approximate number of seeds per gram ^c Number	Approximate number of seeds per ounce ^d Num ber
1		Veronica austriaca L. Hungarian speedwell	0.6	6	3,920	111,130
1		Veronica longifolia L. garden speedwell	0.2	2	16,130	457,285
1		Veronica spicata L. spike speedwell	0.2	2	15,150	429,505
2		Vicia articulata Hornem. monantha vetch	100	500		
7		Vicia benghalensis L. purple vetch	100	500	22	625
2		Vicia faba L. broadbean	500	500		
2		Vicia pannonica Crantz Hungarian vetch	100	500	24	680
2		Vicia sativa L. subsp. nigra (L.) Ehrh. blackpod vetch	50	500	60	1,700
2		Vicia sativa L. subsp. sativa common vetch	150	500	19	540
2		Vicia villosa Roth subsp. varia (Host) Corb. winter vetch	100	500	25	710
2		Vicia villosa Roth subsp. villosa hairy vetch	75	500	35	1,020
7		Vigna angularis (Willd.) Ohwi & H. Ohashi adzuki bean	200	500	11	310
7		Vigna radiata (L.) R. Wilczek var. radiata mung bean	100	500	24	680
		Vigna unguiculata (L.) Walp. subsp. sesquipedalis (L.) Verdc. yard-long-bean		<i>nguiculata</i> (L.) alis Group	Walp. subsp. <i>u</i>	nguiculata
7		Vigna unguiculata (L.) Walp. subsp. unguiculata Sesquipedalis Group yard-long-bean	300	500	8	225
7		Vigna unguiculata (L.) Walp. subsp. unguiculata Unguiculata Group cowpea, southem-pea	300	500	8	225
6		Viola cornuta L. viola	3	30	880	24,948
6		Viola tricolor L. pansy	3	30	735	20,837
1		Vitis vulpina L. riverbank grape	80		32	900
12	с	Vulpia spp. small fescue	-	-	-	-

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Table 6A. Methods of testing for laboratory germination

Kind of Seed	Substrata ^a	Tempera- ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Dormant seed ^f
Cyclamen africanum cyclamen	P, T	20	14 ^b	28 ^c	Good moisture supply	
Cynara cardunculus artichoke and cardoon	B, T	20	7	21		
Cynodon dactylon var. aridus giant bermudagrass	Р	20-35	7	21	Light; KNO3; see sec. 6.9a	Prechill at 10°C for 7 days and then test at 20-35°C. Continue tests of hulled seeds for 14 days and of unhuller seeds for 21 days
Cynodon dactylon var. dactylon bermudagrass	Р	20-35	7	21	Light; KNO3; see sec. 6.9a	
Cynoglossum amabile Chinese forget-me-not	TB, T	20-30	4	10 ^c	Light; some stocks require KNO3	
Cynosurus cristatus crested dogtail	Р	20-30	10	21	Light	Prechill for 3 days at 5or 10°C
Dactylis glomerata orchardgrass	P, TS	15-25	7	21	Light; germination more rapid in soil	Prechill at 5 or 10°C for 7 days and test a 15-25°C
Dahlia spp. dablia	TB, B, T	15	4 ^b	14 ^c	Sensitive to drying in test	
<i>Dalea purpurea</i> Vent, purple prairie clover	T, B	20-30	7	14	Prechill 7 days at 5C. See sec. 6.9c. Hard seeds: see sec. 6.2d and 6.9m(6)	
Daucus carota subsp. carota Queen Anne's-lace	тв	15	7 ^b	21 ^c	Light. Low germination may be due to the presence of embryoless seeds; see sec. 3.2e, PSU# 11	
Daucus carota subsp. sativus carrot	Т, В	20-30	6	14		
Delphinium cardinale cardinal larkspur, perennial delphinium	TB, B, T	15	12 ^b	28 ^c	Very sensitive to warm temperature	
Delphinium elatum Pacific giant, gold medal, and other types belladona, bellamosum types Chinensis types	TB, B, T TB, B, T TB, B, T	20-30 20 20	8 ^b 7 ^b 7 ^b	18 ^c 16 ^c 18 ^c	Sensitive to drying in test	
Deschampsia cespitosa tufted hairgrass	тв	20-30	7	21		KNO ₃ , 5 day prechi at 5-10°C. Ungerminated seeds see sec. 6.2f and 6.9m.

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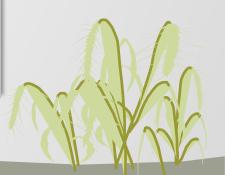


Table 6A. Methods of testing for laboratory germination								
Kind of Seed	Substrata ^a	Tempera- ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Dormant seed ^f		

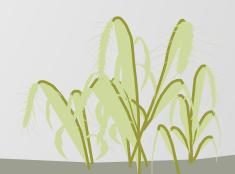


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				J 8

Kind of Seed	Substrata ^a	Tempera- ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Dormant seed ^f
Lupinus albus white lupine	B, T	20	3		Hard seeds: see sec. 6.2d and 6.9m(6)	

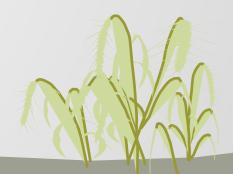
Kind of Seed	Substrata ^a	Tempera- ture (°C)	First count (days)	Final count (days)	Specific requirements and notes	Dormant seed ^f
Tetragonia tetragonoides New Zealand spinach	Т	15; 20	5	21	Soak fruits overnight (16 hr), air dry 7 hr; plant in very wet towels. Do not rewater unless later counts exhibit drying out	On 21 st day scrape fruits and test for 7 additional days

Table 6A. Methods of testing for laboratory germination





-AOSA, ISTA, FSA; 1960-1975 Rules used by most of the world





Seed Testing-2

What explains this huge increase???





AOSA+, FSA, Canadian M&P, ISTA+

Present Rules

Large increase in types of tests

Moisture content, cultivar purity, TZ, vigor, etc.

> Approximately <u>700</u> species

>More than one method for the same test (some species)

- Artificial substrate used to be cotton sheets/blue blotter
- Now 10 substrates plus soil

Regulatory labs must be familiar with all the Rules for all the listed species



Example: fava beans sample, with different germination test results (range of 17%) by three labs, using different Rules (ISTA, AOSA, Canadian M&P), substrates and temperatures.



Job Description:

- Analyze corn and soybean seed samples for the following tests:

- Accelerated aging
- Warm and cold germinations
- Purity analysis
- Inert/foreign material
- Noxious weed identification

- Compile and analyze data from seed testing and provide feedback to seed production team.

- Work within the seed production team to audit quality concerns, identify quality issues and create processes for quality improvement

- Audit current processes and make changes as needed on an annual basis. Create new processes, including the development coner methodologies for seed testing, as required to achieve the objectives of the lab.



JOB REQUIREMENTS

- 1. Education and Training:
 - Bachelor Degree
 - Registered Seed Technician is preferred or ability to obtain RST accreditation in less than two years.
- 2. Technical Requirements:
 - Excellent verbal and written communication skills.
 - Possesses knowledge of various seed testing methods for corn, soybeans and wheat is preferred.
 - Possesses a positive attitude.
 - Ability to establish priorities, work independently, work as a team member, and proceed with objectives without supervision.
 - Possess a high-level attention to detail, organized, and be able to consistently deliver timely and accurate results.
 - Proficiency with computers, Microsoft Office and spreadsheets.

What are the added costs of maintaining the ability to test and apply all the Rules?

- Significant but not substantial
 - Thousands of dollars annually for the different Rules
 Inefficient use of resources (space/equipment)



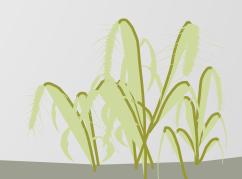


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What are the added costs of maintaining the ability to test and apply all the Rules?

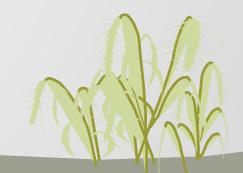
- Significant but not substantial
 - Thousands of dollars annually for the different Rules
 Inefficient use of resources (space/equipment)
 A wide range of planting materials
 Other reference materials



Tests and Re-Tests!

As a regulatory lab we often test and re-test under different conditions.

- *We have to get it right!
- Whatever we report is considered the true evaluation of the seed lot.



CA Food and Agricultural Code. 52363(b): "A written report which is issued by the State Seed Laboratory that shows the analysis of any such sample is prima facie evidence of the true analysis of the entire lot from which the sample was taken."



Tests and Re-Tests !

As a regulatory lab we often test and re-test under different conditions.

- *We have to get it right!
- Whatever we report is considered the true evaluation of the seed lot.
- Not every sample gets re-tested
- >Added costs of testing, re-testing and investigations: Time!

Do All Regulatory Labs Retest?

Some, but not all regulatory labs!

Our policy (germination): a label is in compliance until proven otherwise

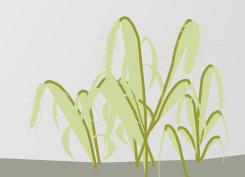
> At least two tests under same or different conditions, and no single test produces results indicating label is in compliance

Different Conditions

- Replant using other options in Rules (substrates, temp., etc.)
- Replant after removing seed treatment
- Replant under grow lights/growth chamber
- Add germination promoting chemicals
- Disinfect seeds
- Test viability by TZ

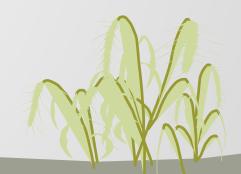
Retesting/Multiple Testing Frequency

- About 8% of all regulatory samples for germination
- Service: about 5% of all samples for germination
- ➢Investigative: every sample
- Time of actual testing can be more than 10x 'normal' sample testing time (5x on average)



Treated Squash Sample Retest-Germination Label Violation

Planted according to Rules
Planted according to Rules (repeat test)
Planted in sand-extended test
Planted in peat pellets-extended test



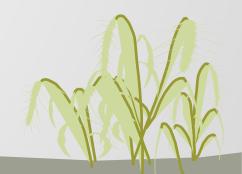
Investigative Sample-Fava Beans

Planted according to ISTA Rules Planted according to Canadian M&P Rules Planted according to FSA/AOSA >Planted in peat pellets Seeds planted with and without disinfection with bleach Germination and abnormal types determined for each planting method, separated into root and shoot abnormals

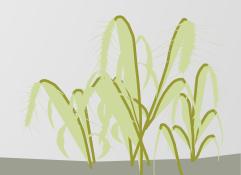
Testing a 'New' Species

Part of SL SOP (based on previous experience)

Plant according to most common method in Rules
 Plant using alternative methods
 Plant in soil/peat pellets
 Based on species, determine need for dormancy breaking treatments



Fees for Investigative Samples?





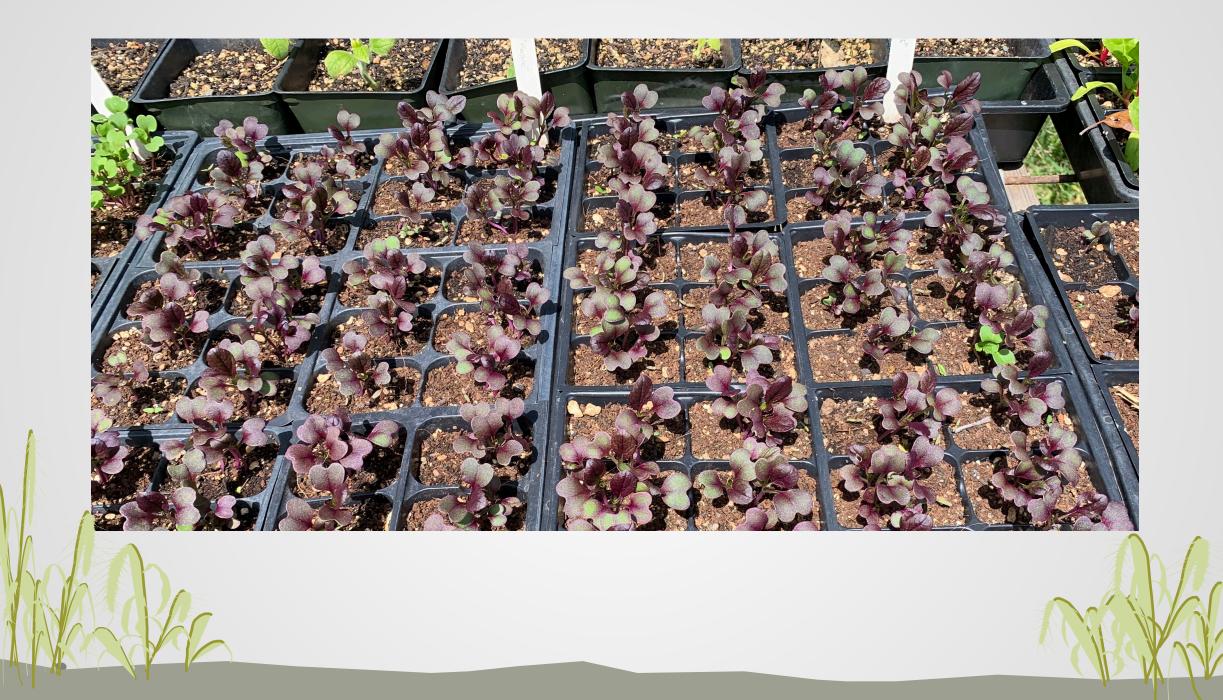
In-house training of Environmental Scientists and Associate Seed Botanists

- Training of analysts from other USA labs (mostly for certification)
- Training workshops
 - Purity Vigor testing Germ. diagnostics

Germ. Methods Moisture testing Seedling Evaluation Referee testing Statistics in seed testing



- Seeds of most species do not behave 'normally' when germinated on artificial media
- Many things can go wrong during a germination test, unrelated to seed quality
- Variation in germination testing remains high for many reasons





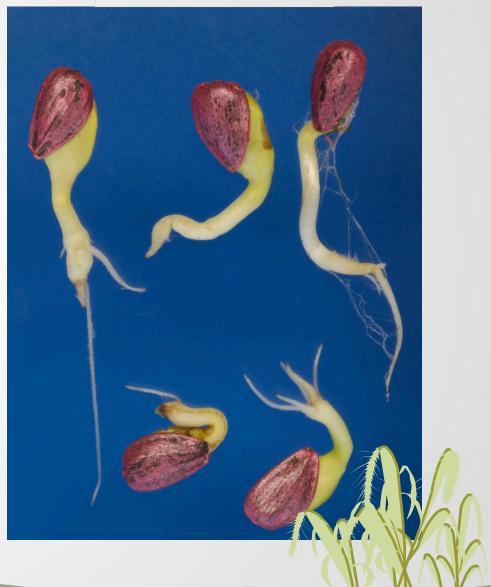




Safflower









Purity violations are always more common than germination violations

*****Tolerances are wider for germination

*Tolerance: maximum expected difference due to <u>chance</u> "A larger and more arbitrary tolerance must be allowed in the results of germination tests [compared to purity results]." USDA, 1928.

Label: 85%; Test: 78% = results are in tolerance

Method Validation-1

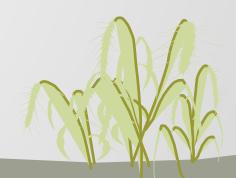
- Validating suitable methods for germination testing and seedling evaluation is as critical as good training
- The impact of proposed new rules on the CA seed industry is always a consideration



Method Validation-2

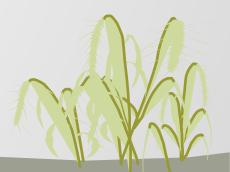
- Modifications of existing methods
 Additions of test options
 Selecting best methods for 'new' species
- Referee Studies Method Validation

Introduction of new methods (research)



Benefits to the Seed Industry

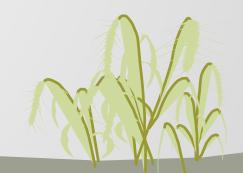
- ✓ Reliable evaluations through rigorous protocols
 ✓ Training
- ✓ Research
- ✓ Information
- ✓ Validation (referee) studies and Rules proposals
- Involvement in different AOSA and ISTA activities to promote uniformity within and across organizations.





Reduced period of tall fescue germination test (21 to 14 days)

- Reduced germination period of radish germination test (7 to 6 days)
- >Identification of species with hard seeds



Examples-2

- Improve ROA (report of analysis) for transparency and accuracy (ongoing); <u>ASTA request</u>
- Review accuracy of florescence levels in determining annual/perennial ryegrass (initiated)
- Improve accuracy of seed counts for flower/wild species
- Development of testing and evaluation protocols and reference website for wild/native species (ISTA Technical Working Group)

ASTA and Testing Uniformity

- The American Seed Trade Association (ASTA) and the Association of American Seed Control Officials (AASCO) resolved to investigate the uniformity of seed lab analyses
- Blinded samples of known composition (9 species) were sent to several labs (regulatory and commercial)
- I was asked to provide statistical analysis, interpretation and recommendations



Results-1

- In general, purity analyses results were consistent among labs
- Germination results indicated significant variation among labs for many species. Differences in results among labs were as high as 36%
- TZ test results also varied. Differences among labs for the same tested species were as high as 60%



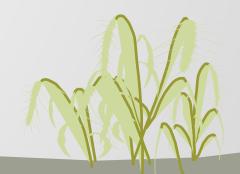
Results-2

- Significant deficiencies in the ability of labs to detect and classify noxious weeds, weeds and other crop seeds.
- Significant deficiencies in the ability of labs to correctly identify foreign seeds
- A large number of determinations of foreign seed numbers were significantly lower than 'spiked' results. In many cases, contaminants were entirely undetected.

ROA compliance was inconsistent

Recommendation

- Concerned organizations (AOSA and SCST) should resolve to improve uniformity in all areas of seed testing, as well as the content of official seed test reports
- >ASTA asked AOSA to work on improving test uniformity!



- AOSA and SCST members formed several working groups (WG)
- The CA Seed Lab is represented in several WG and leading the effort in the two most critical areas: purity and germination testing
- Germination WG: Seedling Evaluation Reference and Training Database



Seedling Evaluation Database

Funded by the USDA; a <u>5-year</u> project.

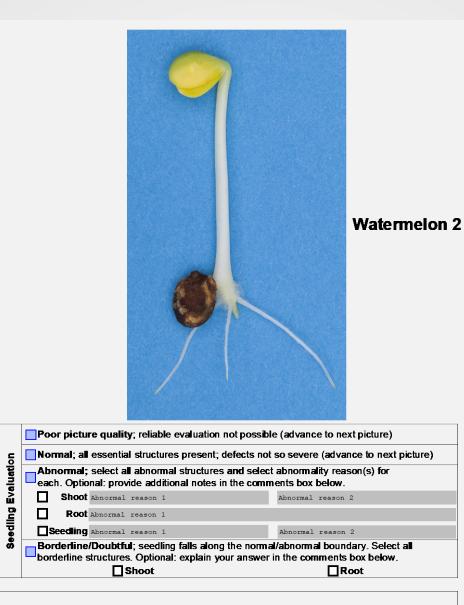
Much more than a database; a systemic and quantitative way of understanding and improving seedling evaluation



Seedling Evaluation Database-Objectives

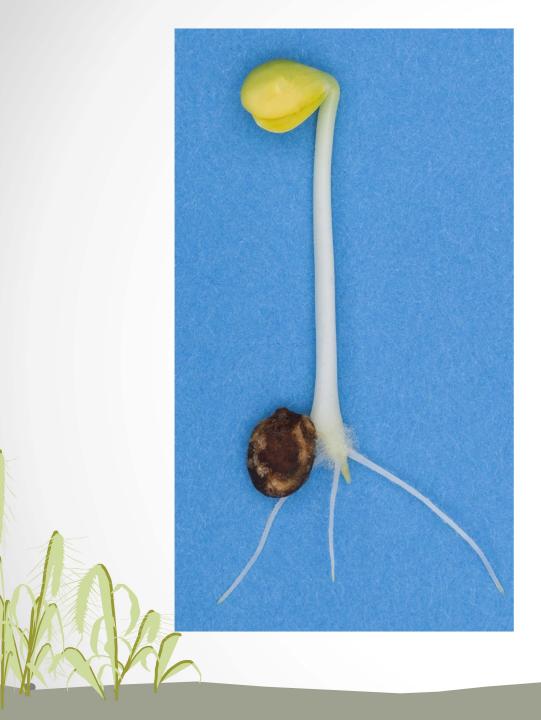
Objectives:

- Understand why analysts' results vary
- > Resolve many of the gray areas related to seedling evaluation
- > Provide uniform training to all analysts (ISTA as well)
- Reduce errors and variation in germination results that can negatively impact on producers/sellers
- > Increase repeatability of evaluations for regulatory purposes



Comments (optional)





Λ



Evaluation	Poor picture quality ; reliable evaluation not possible (advance to next picture)		
	Normal ; all essential structures present; defects not so severe (advance to next picture)		
	Abnormal; select all abnormal structures and select abnormality reason(s) for each. Optional: provide additional notes in the comments box below.		
Ň	Shoot Abnormal reason 1	Abnormal reason 2	
ling	Root Abnormal reason 1		
Seed	Seedling Abnormal reason 1	Abnormal reason 2	
Ň	Borderline/Doubtful; seedling falls along the normal/abnormal boundary. Select all borderline structures. Optional: explain your answer in the comments box below.		

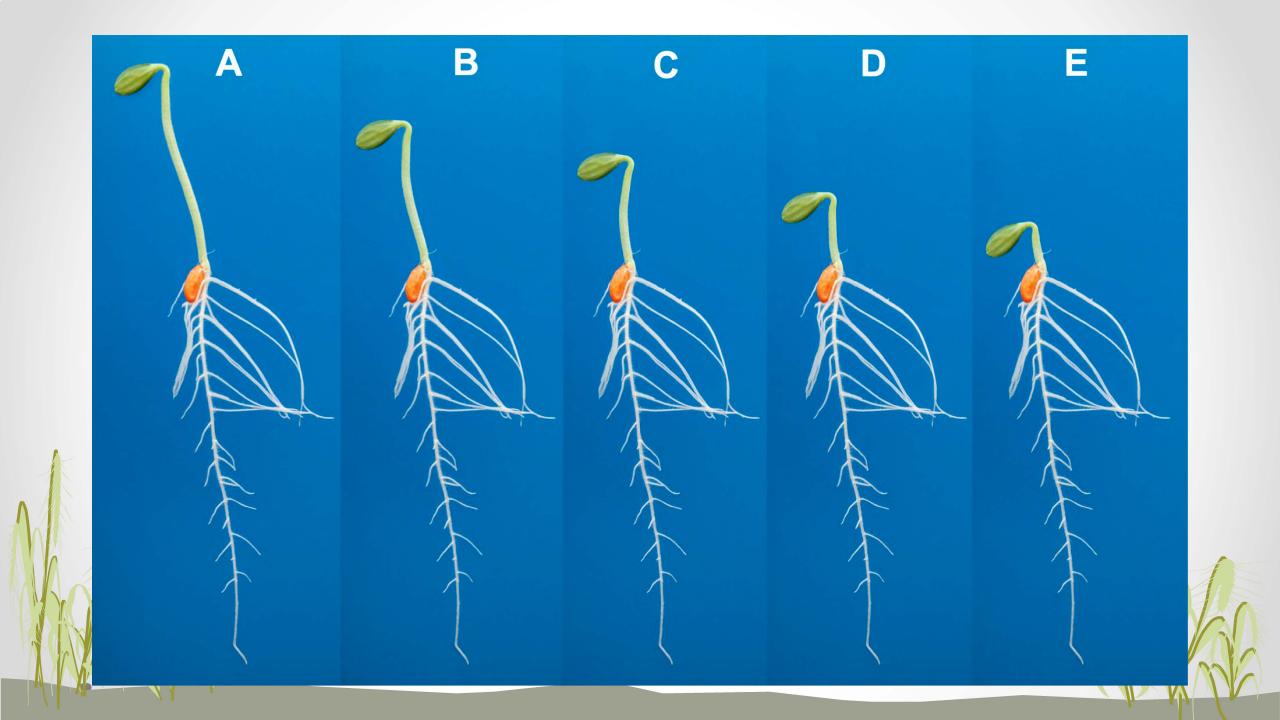
Comments (optional)	

Λ



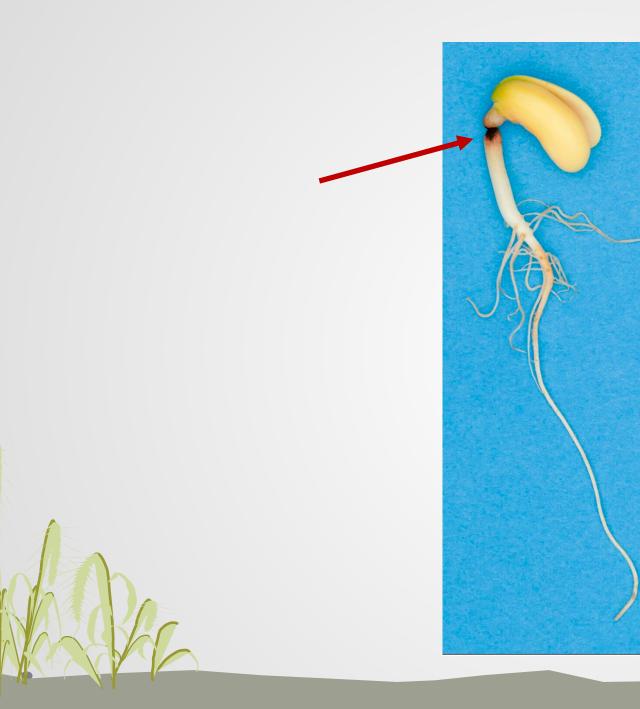




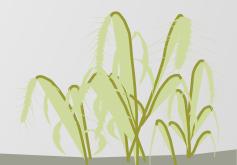




Which is more uniform, rep 1 or rep 2?



Field bean



Thank you!

