Revisiting a Cold Case- Devitalization of Germinated Seedlings by Freezing

Cold Case Narration by Maranda Gillen

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Introduction

- Seedling devitalization methods are important when discussing a process for regulated or stewarded material handling.
- Compliance packets typically list approved methods of devitalization by crop or event, and ultimately adhere to USDA guidelines.
- Both trait providers and labs conducting services need to have confidence in the process that seeds or plants will not propagate or be released in the environment.

- This presentation will reveal a high-throughput method for the disposal of germinated corn, cotton, and soybean material.




Devitalization Methods

Commonly used methods that can be found in compliance packets

- Grinding, heat or steam treatment, incineration

- Other methods that may be listed: composting, bleach treatment, deep burial

Devitalization Methods

Universal challenges of devitalization methods-

- Demonstration of devitalization
- Equipment necessary & calibration
- Routine verification or certification
- Time and resources to conduct devitalization
- Capacity

Devitalization Methods

Freeze Method

- Reduces challenges met with capacity and equipment use
- Designed to be a high throughput method for the disposal of germinated corn, cotton, and soybean
- Method is based on the physiology of corn, soy, cotton plants, and similar methodologies have been researched before-
  - The method comprising the steps of hydrating a viable whole plant seed and freezing the hydrated whole plant seed. https://patents.google.com/patent/US20100037358A1/en
  - Any freeze damage below the cotyledons will kill the plant. https://www.kygrains.info/blog/2020/4/20/assessing-freeze-damage-on-emerged-and-emerging-corn-and-soybeans
  - Freezing temperatures for at least two hours may be enough to freeze and kill the growing point. http://www2.ca.uky.edu/agcomm/pubs/agr/agr192/agr192.pdf
  - Air temperatures of 29 F are necessary to completely kill corn and soybean plants. https://www.agry.purdue.edu/ext/corn/news/articles.02/Frost_Freeze-0520.html

Lab Scene Set Up

In practice, a combination of devitalization methods may be used, based on testing needs.

- ❑ You work in a seed testing lab, depending upon the test method, seed samples could possibly be grown out to evaluate, harvested for tissue, or processed to powder.
- ❑ A customer sends in 20 samples each of regulated canola and corn. 40 Samples Total.
- ❑ Tests include the following methods-
  1. PCR pool testing
  2. Germination testing
  3. Trait Purity testing by ELISA and Herbicide bioassay
- ❑ During sample processing, care is taken to ensure all seeds are accounted for. 
- ❑ Testing is completed 

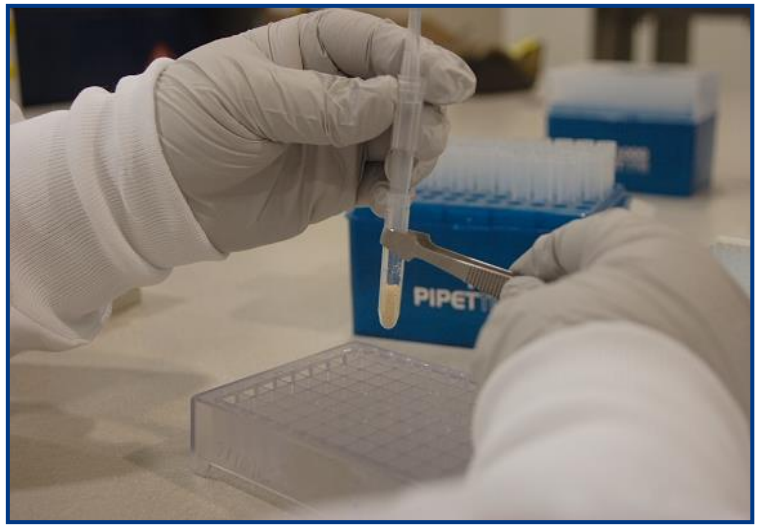
? What does the lab do to dispose the regulated material produced during testing?

Lab Scene Set Up

? What does the lab do to dispose the regulated material produced during testing?

PCR pool testing-

- Grinds seeds in pools to a fine powder. Devitalized? Yes



Lab Scene Set Up

? What does the lab do to dispose the regulated material produced during testing?

Germination- Canola

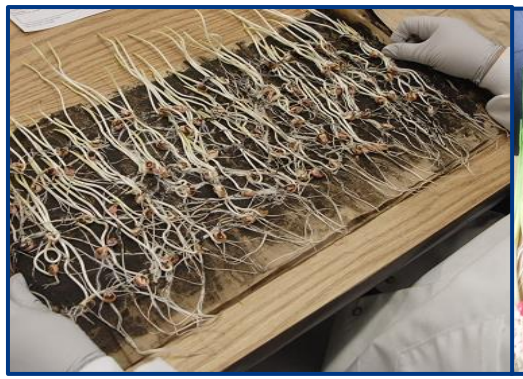
- Lab saves all seed tested. Devitalizes all material by heat treatment.

Lab Scene Set Up

? What does the lab do to dispose the regulated material produced during testing?

Germination- Corn

- Plants & germinates seed 400 seeds or 4 towel reps of 100 seeds per sample.
- Removes & saves un-germinated seed during evaluation.
- Seed that did not germination- Devitalizes by manual destruction/grinding/autoclaving.
- Lab saves the evaluated germinated material in bags to devitalize. ~80 germination towels worth of seeds or 8,000 seedlings.

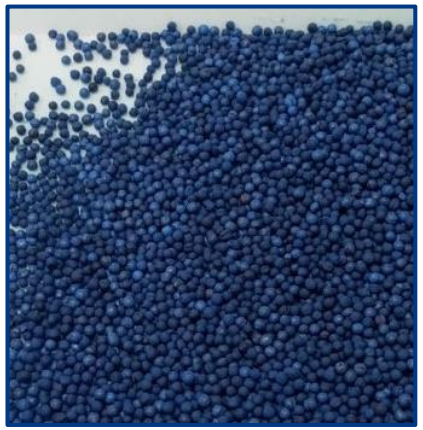


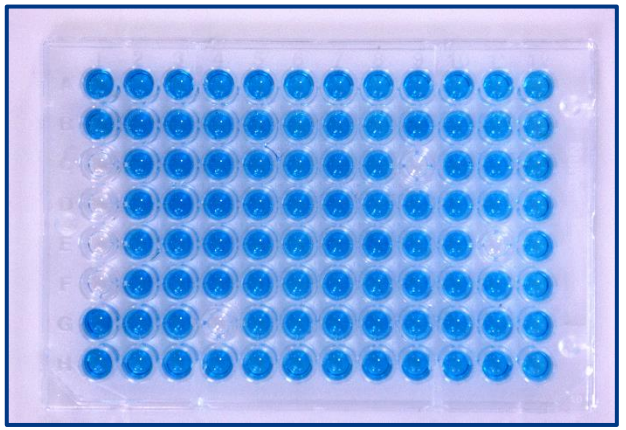
Lab Scene Set Up

? What does the lab do to dispose the regulated material produced during testing?

Trait Purity- ELISA

- Conducted trait purity testing on the canola samples by ELISA.
- A seed pulverizing technique is done as part of the extraction method. Devitalized? Yes





Lab Scene Set Up

? What does the lab do to dispose the regulated material produced during testing?

Trait Purity- Herbicide bioassay

- Conducted trait purity testing on the corn samples by Herbicide bioassay-
- Plants & germinates 400 seeds per sample, 8 towel reps of 50 seeds/sample.
- Lab removes & saves un-germinated seed during evaluation.
- Seed that did not germination- Devitalizes by manual destruction/grinding/autoclaving.
- Lab saves the evaluated germinated material in bags to devitalize. ~160 germination towels worth of seeds or 8,000 seedlings.

Lab Scene Set Up

- What about the germinated material produced in germination and herbicide bioassay testing?
- Enter Freeze Method for Devitalization

- Total Germinated seedlings produced during germination and herbicide bioassay evaluation & testing was 240 towels of seedlings or 16,000 seedlings to devitalize.
- This could be a couple weeks of grinding or autoclaving, or just a few bags to place in a freezer for 3 days.

Freeze Method Purpose & Summary

- A high-throughput method for the devitalization of germinated plant material was pursued, so there would be an alternative method to autoclaving regulated plant material.
- The results from the validation design concluded that corn, cotton, and soybean are devitalized after a 3-day period in the -20^{o}C chamber.
- All regulated material handling procedures were adhered to during the validation of the devitalization method. Only non-regulated material was used in the design.

Validation Procedure

1. Non-regulated samples were evaluated, labeled, then placed into 5 gallon trash bags. Sample bags were then stored to be frozen in a -20C chamber.
2. Samples were taken out of the cold chamber approximately 72 hours afterwards and allow to defrost.

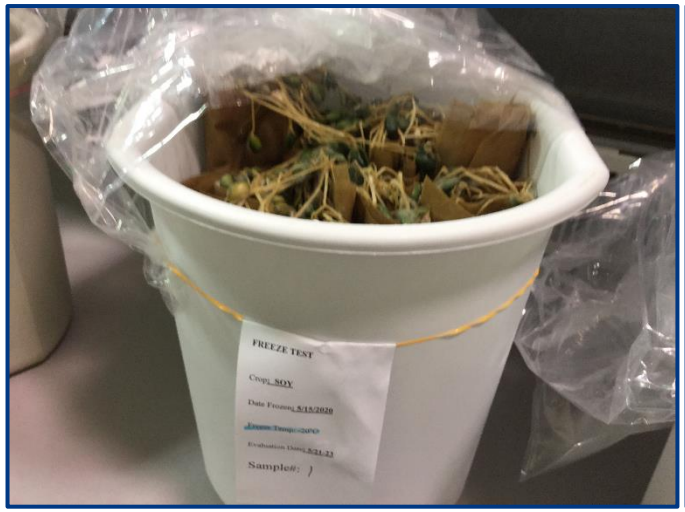
5 gallon trash bag (1 ft 11-15/32 in x 2 ft 1-3/8 in or 59.6 cm x 64.5 cm).

Trash bag fill-weight limit is 3200g or ~35 rolled towels.

Validation Procedure

3. Defrosted samples were then placed into buckets, given additional water to facilitate growth, and stored in the 25C light chamber.
4. After 2 days, crops were evaluated for vigor and vitality, if the results were not definitive then the length placed in the 25C light chamber was extended.
5. Pre and post devitalization evaluation was conducted by trained staff as guided by RSTs

Soybean samples froze 72 hours, then stored 2-3 days in light chamber



Results- Images

Soybean After 72 Hours in -20C Chamber

Germinated soybean after exposure from -20C chamber for 3 days. Sample was then placed in 25C light chamber to stimulate growth for vitalized seedlings. Significant freeze damage in root and shoot of plant depicted by wetness, yellowing, and lack of structure revealing cellular damage.

All seedlings deemed devitalized.

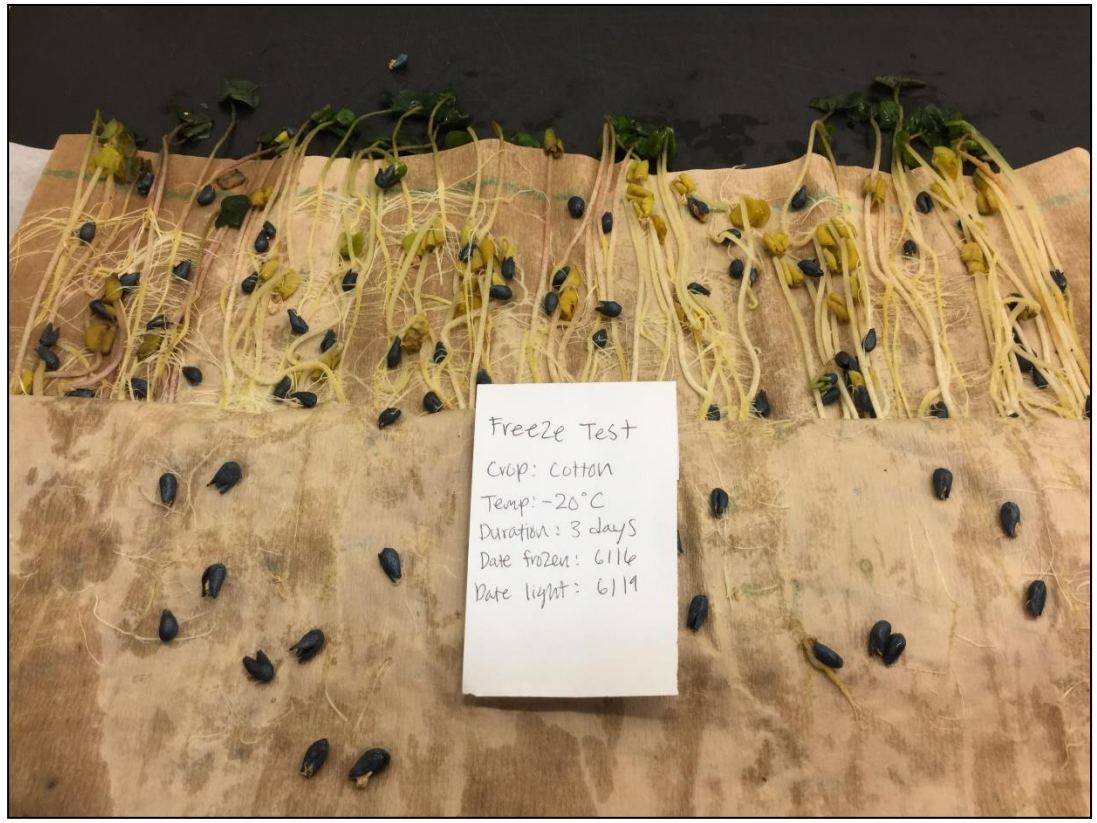
Method validated for use.

Soybean After 96 Hours in 25C Light Chamber

Germinated soybean after exposure from 25C light chamber for 4 days after freezing. Sample reveals advanced decay and lack of vitality. Soy structures such as the cotyledons, radicle and the first true leaf reveal irreparable damage.

Results- Images

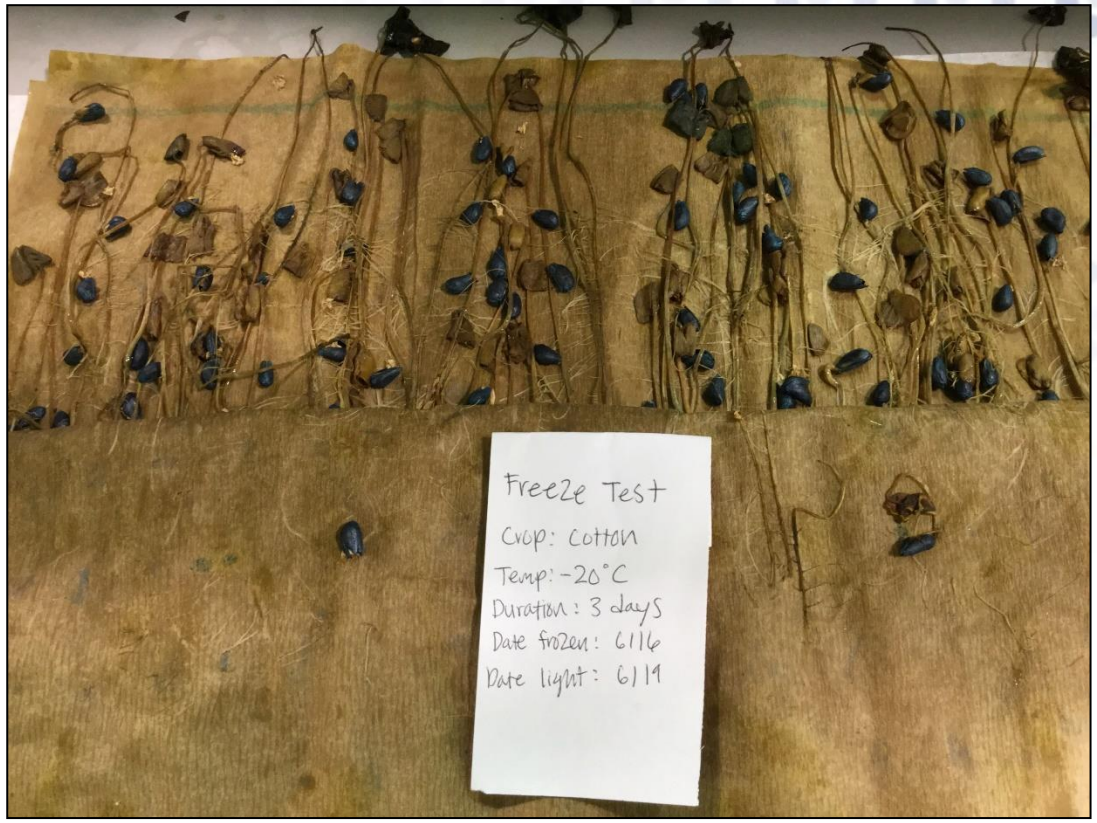
Cotton After 72 Hours in -20C Chamber



All seedlings deemed devitalized.

Method validated for use.

Cotton After 48 Hours in 25C Light Chamber



Germinated cotton after exposure from -20C chamber for 3 days. Patches on the wet towels as well as damage to morphology reveal significant cellular damage to the seedlings. Sample was then placed in 25C light chamber to stimulate growth for vitalized seedlings.

Germinated cotton after exposure from -20C chamber for 3 days as well as 25C light chamber for 2 days. Wilting and pigmentation changes in roots and shoots reveal extreme decay.

Results- Images

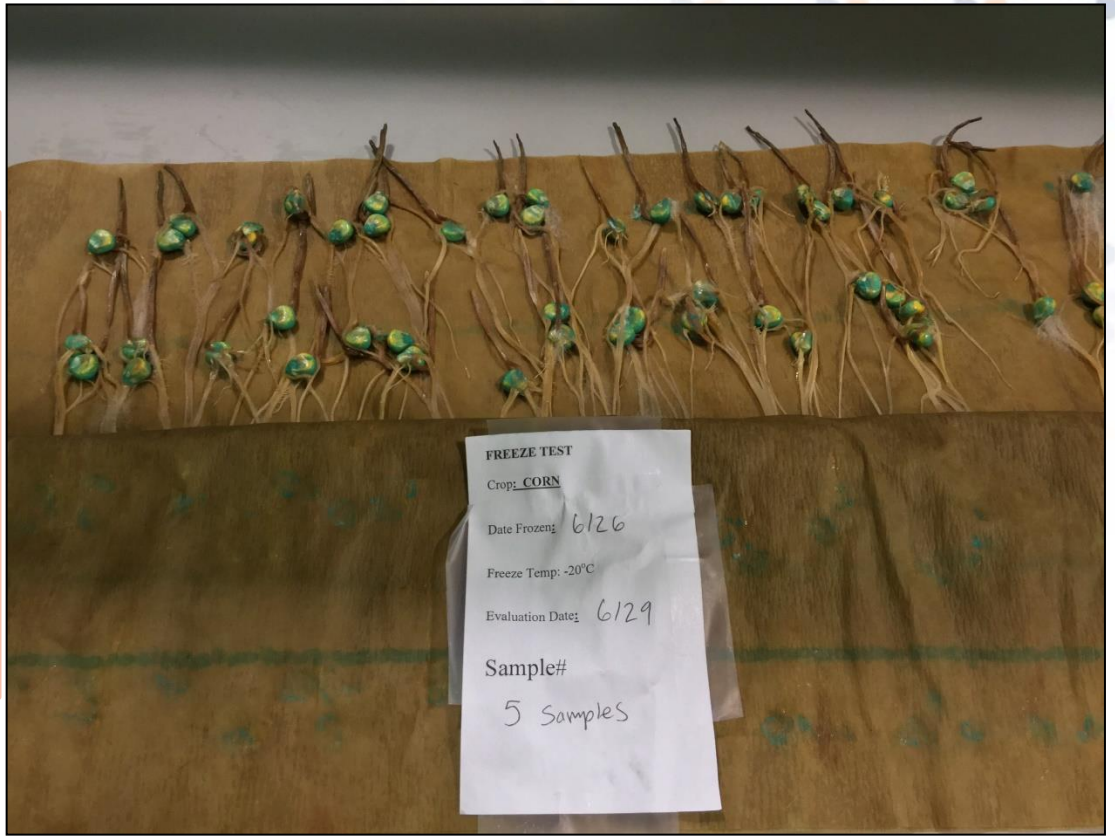
Corn After 72 Hours in -20C Chamber

Germinated corn after exposure from -20C chamber for 3 days. Sample was then placed in 25C light chamber to stimulate growth for vitalized seedlings. Significant cellular damage in root and shoot of plant depicted by ice crystals on shoots and roots.

All seedlings deemed devitalized.

Method validated for use.

Corn After 48 Hours in 25C Light Chamber



Germinated corn after exposure from -20C chamber for 3 days as well as 25C light chamber for 2 days. Wilting and pigmentation changes in roots and shoots reveal decay. Significant damage to the seed.

Conclusion

- The samples that were determined to be devitalized were then disposed of in the garbage.
- All three crops were successfully devitalized in 3-days, in bulk quantities of ~35 rolled towels per bag in a -20C chamber.
- Hard/un-germinated seeds will need to be removed and devitalized in a grinder, autoclave, or other alternative method for physical destruction.

Support from the Industry

- Presented by Brenda Johnson 
- AEIC –Soliciting for participating labs to conduct multi-lab study
- "*The SCST Genetic Technology Committee supports the research into devitalization by freezing feeling that it is a valuable and useful effort intended to provided further options and techniques in the field of seed testing*." Molly Richeson, RGT, SCST Genetic Technology Committee Co-Chair
- Current status of movement through ASTA in support of submission to USDA / Seed Science Foundation proposal

Questions & Feedback

Thank you!