Cold Testing Options in Vigor Testing of Seeds

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- What is Seed Vigor?
- Available Vigor Tests
- Cold Tests
- Determining WHC
- Tray Method
- Rolled Towel Method
- Shoe (Deep) Box Method
- Saturated Method
- Controls (Check Samples)
- Vigor Testing Tolerances
- What Do Results Mean?
- Cold Tests and Field Emergence
- Importance of Proper Sampling
- Resources

Standard Germination

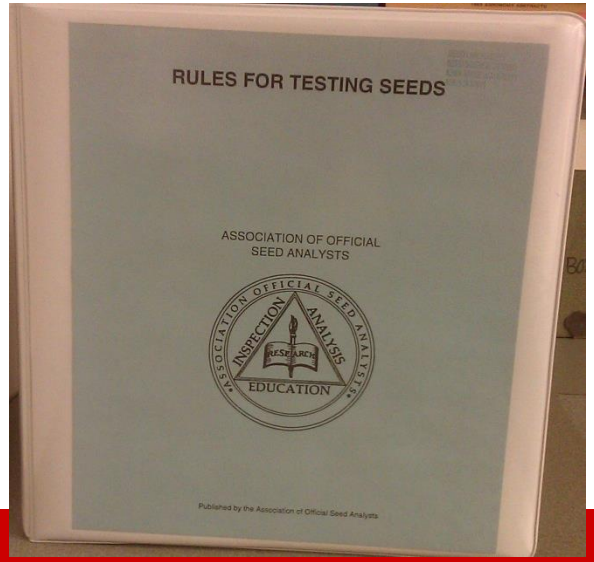
Ideal temperature and moisture for seeds.

Standardized. Reproducible within tolerances - within and between labs.

May have high correlation with the field if field conditions (temperature, moisture, absence of pathogens) are favorable.

Volume 1: planting information, tolerances

Volume 4: seedling evaluation criteria



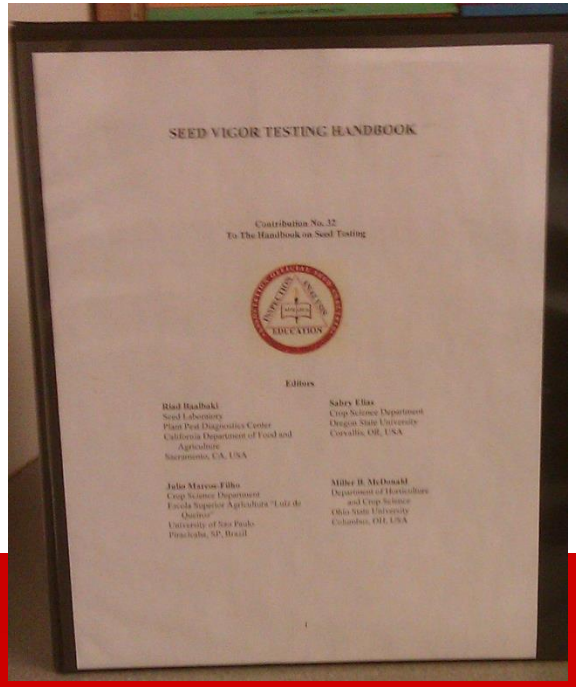
Seed Vigor

- **AOSA**: those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions.

- **ISTA**: Seed vigour is the sum of those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence.

AOSA Seed Vigor Testing Handbook

ISTA Handbook of Vigour Test Methods



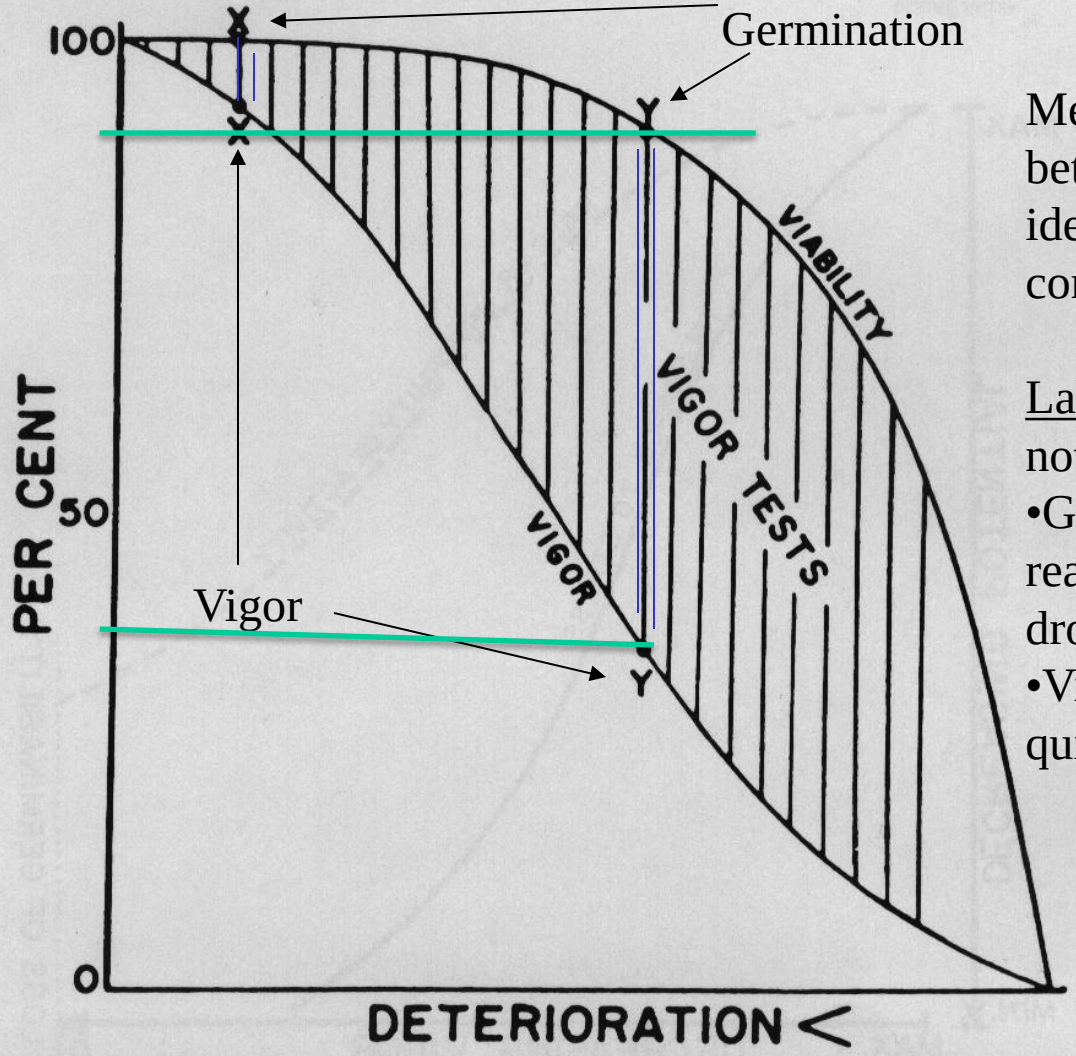


Figure 2. Relationships among seed vigor, viability (germinability) and deterioration, and the area for application of vigor tests. The X and Y points on the viability and vigor curves illustrate the increasing "gap" between germinability and vigor with increasing deterioration. From Delouche and Caldwell (1960).

From AOSA Vigor Handbook

Measure the difference between testing under ideal and unfavorable conditions.

Labeling: Warm germ, not vigor.

- Germ more apt to stay reasonably level or drop slowly
- Vigor can drop very quickly

Vigor Tests

- Accelerated Aging
- Cold (Shoe box, tray, towel, saturated)
- Electrical Conductivity
- Cool Germination (cotton)
- Seedling Vigor Classification
- Shoot/root weight
- Soak test
- And more
- Use of restriction fragment length polymorphisms (RFLPs) to identify and map quantitative trait loci (QTL) underlying seedling vigor-related traits[2]

Saturated Salt AA
Tetrazolium
Seedling Growth Rate
Speed of Germination
Controlled Deterioration
Potassium Leakage
Computer Imaging

Direct vs Indirect Vigor Tests

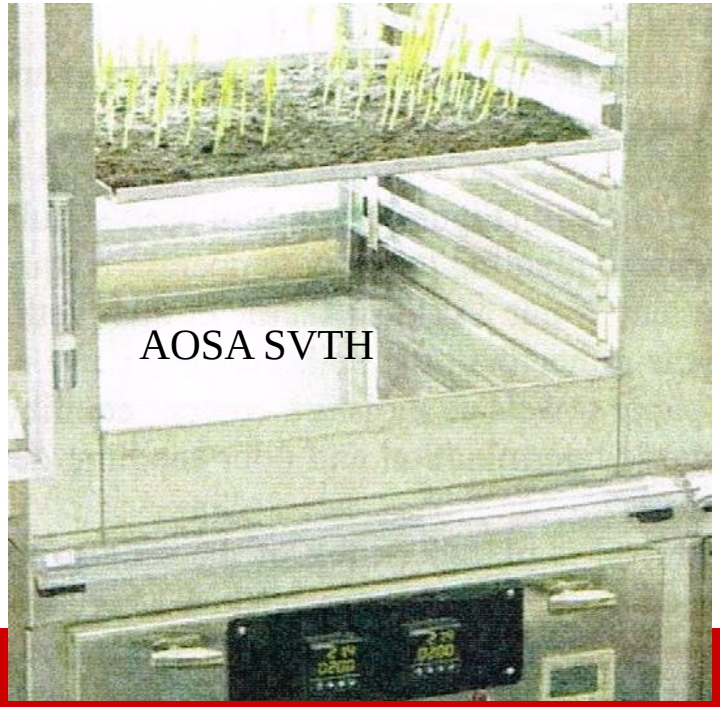
Direct: those in which an environmental stress expected in the field is reproduced in the lab and the percentage & rate of seedling emergence is recorded (**e.g. cold test**).

Indirect: those in which other characteristics of the seed which have proved to be correlated with an aspect of field performance are measured (e.g. respiration rate, conductivity test).

Cold Test

- The cold test is generally regarded as the standard vigor test for temperate field and sweet corn hybrids (p.67 SVTH).
- Simulates early spring field conditions by providing high soil moisture and low soil temperature (and possibly pathogen activity)
- Oldest and most extensively evaluated vigor test method in the United States.
- "Shoe (Deep) Box", Rolled Towel, Tray, Saturated, Extended

P. 174 Crops for which the cold test was able to detect vigor differences … .



Tray Method

- Typically crepe cellulose paper (TC) under covering material (sand/soil, sand, ?)
- Recommended that amount of water added brings "covering material" to 70% WHC. Match level of covering material to amount of water added.
- 10°C recommended for cold period
- Length of time in cold, warm temperatures varies:

(7/4, 3 d cold/4 d warm) 7d cold/7d warm (10/7, 14/7), … .

- If soil present, check for pythium? Check for virulence?
- Soybeans will bubble the surface during seven days in the cold room at 10°C

Determining Water Holding Capacity

- Conducting the tray cold test requires determination of the water holding capacity of the covering material (sand, sand/soil, soil, etc.).
- This procedure is on **pages 30-33** of the AOSA Vigor Testing Handbook.
- According to soil scientists at Iowa State there are at least two potential problems with determining WHC:
  - The depth of the covering material in the WHC test should mirror that of the cold test.
  - Determining the WHC of the covering material doesn't account for the WHC of the crepe cellulose paper.

Determining Water Holding Capacity

Determine how much sand/soil is placed on cold test trays.

Weigh all components (tray, cheesecloth, sand & soil mix)

Use tray with multiple holes big enough to allow drainage.

Place cheesecloth on the tray to prevent sand & soil from being lost.

Place sand/soil mix from step one evenly over cheesecloth.

Determining Water Holding Capacity

Preferably add water from bottom of tray until mix is saturated

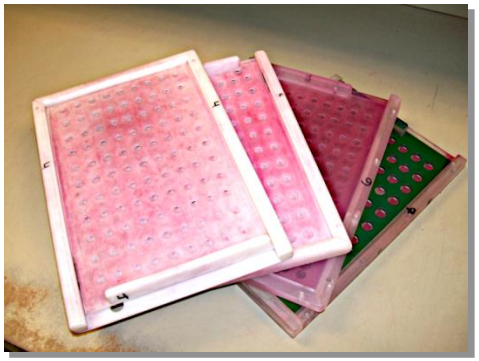
Place tray in germination cart and allow to drain. Place wet tray at bottom of cart and shut door.

After tray stops draining weigh tray. This is 100% WHC. Calculate 70% of this amount.

Tray Cold Test – ISU Procedure

- Place one sheet of 12-ply Versa-Pak™ on each tray and add 1100 mL of water via a watering table. Mark door tag with time and date of "watering".
- Place trays in cart overnight in 10°C cold room.
- Plant two samples per tray (200 seeds per sample). Press down seeds.
- Add 4:1 sand/soil mixture. Remove excess with leveling board (to achieve 70% WHC).
- Complete door tag. Move cart to 10°C cold room (without light).
- Seven days later, move cart to 25°C with exposure to vertical lights to rear of cart.
- Evaluate samples on day 5-7* according to the modified AOSA Rules.
- Run one or two check samples. Results of controls should be within two standard deviations of known mean.

Starting the test



- Water trays and chill at 10°C overnight.
- To maintain exposure to cold temperature: Remove one tray at a time? Place towels on cold plate? Chill covering material?
- Because test isn't for labeling, most labs plant 200 seeds per sample. Some customers request 400 seeds.
- Some labs place reps from a sample on one tray, while others may place reps on different trays within the same cart or in different carts.
- Seeds should be made to lay flat to reduce chance of being scalped off during covering.

Mixing Sand & Soil

- The finer the sand, the more care is needed not to overwater.
- If soil is used, it must be consistent during a testing season and hopefully over multiple seasons.
- Soil is sieved to remove clods, but it doesn't have to be fine.
- Transporting and handling soil and sand can be difficult.

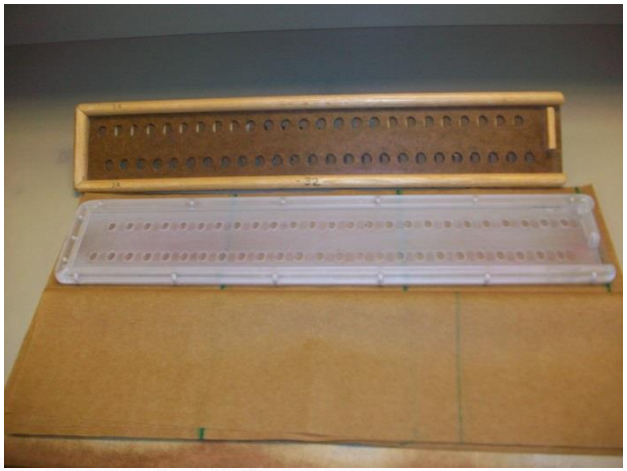
Covering Trays

- A wooden frame is useful to allow excess sand/soil mix to fall into wheel barrow.
- Sand/soil must be applied and then excess removed quickly to prevent caking.
- But care must be taken to prevent samples from being mixed or reps from a sample from being mixed.

What Type of Covering Material?

- It is desirable to use soil in the tray test.
- Are the benefits of using soil in a cold test outweighed by the variability that may come with it?
- Soil can be linked to variability within a lab as well as between labs.
- The type of soil may vary greatly from lab to lab. Within a lab the type of soil should be consistent, but presence and virility of pythium can vary.
- One option is to grow pythium on oatmeal agar, dry and shred the agar, and blend it into the soil.

- Store covering material and moist towels at 10°C
- Plant two rows of 25 seeds, 6 (2.36”) & 12 cm (4.7”) from top of towel
- Use three towels (one above seeds, three below)
- Add just enough sifted soil to make contact with seeds
- Seven days at 10°C, seven days at 25°C
- Soil. Or soil & sand, peat, or vermiculite.
- Least bulky cold test?



Rolled Towel Cold Test

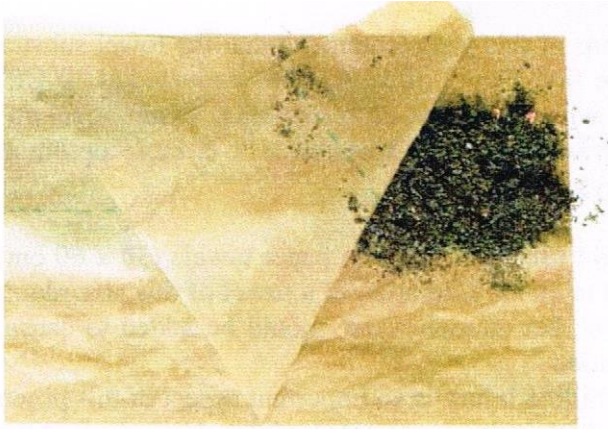


Figure 4.18 Seeds are planted on two moistened paper towels, covered with a thin layer of soil and a third cold, saturated towel is placed over the soil and seeds prior to rolling (Photograph from Riad Baalbaki).

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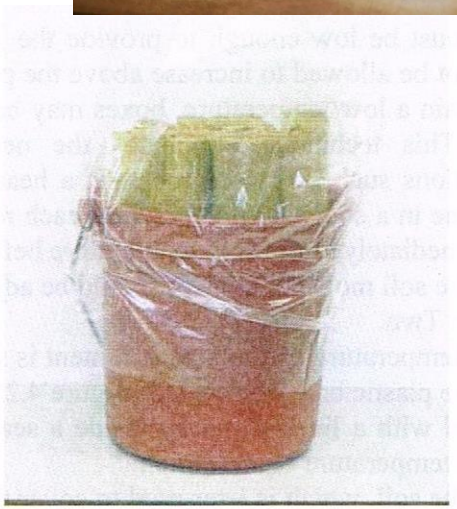
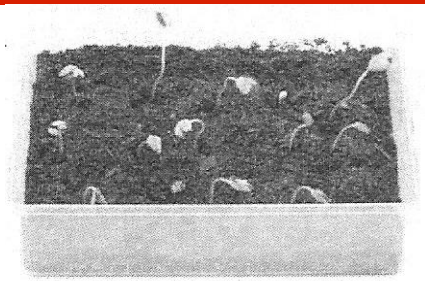
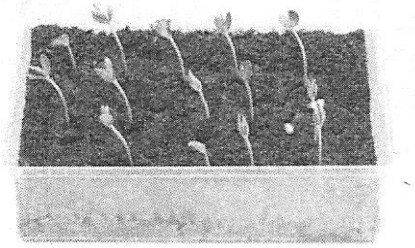


Figure 4.19 Rolled towels containing soil and seeds are placed in a bucket and covered with a plastic bag to retain moisture prior to placement in a cold room (Photograph from Riad Baalbaki).

Shoe (Deep) Box Cold Test



SVTH



- Store moist soil at 10°C
- Place 2.0 cm (~3.4”) layer of soil into box
- Place 50 seeds on soil and gently press in
- Add 2.0 cm of soil
- Determine 70% of water holding capacity before planting
- Seven days at 10°C, four days at 25°C

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ISU

Saturated Cold Test

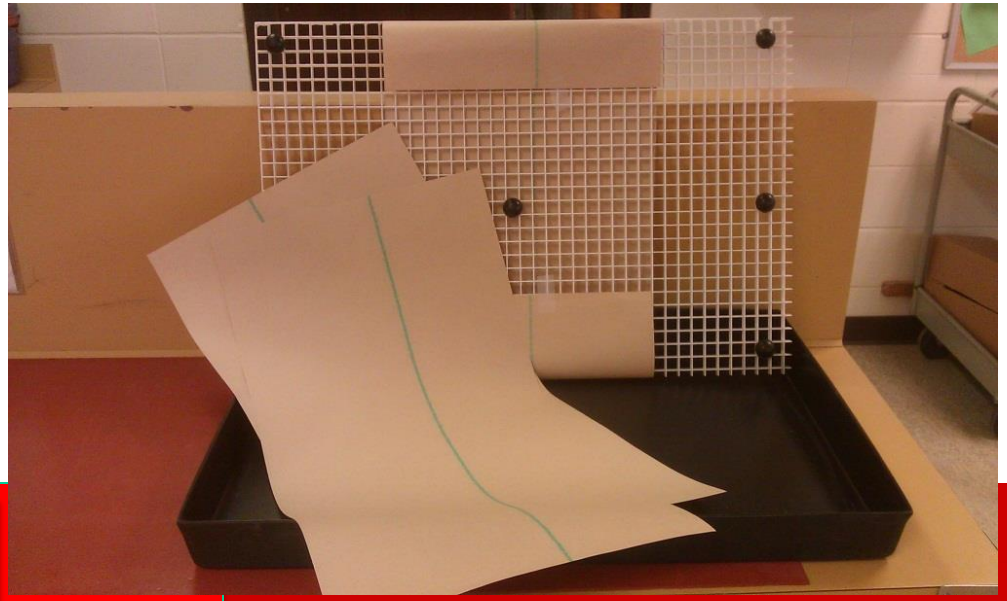
- Higher moisture level (saturated)
- More stress due to higher moisture (plus even more if seeds are pressed into thicker layer of soil)
- Corn seeds turned embryo-side toward soil or left as is?
- Looking for “finer” differences between seedlings than in other cold tests (because of only 3-4 days growth)

Saturated Cold Test

- Use sifted soil.
- Depth of soil: enough to cover towel or enough to reduce oxygen
- Primarily used for corn. Also for sugar beet, tomato, and rice.

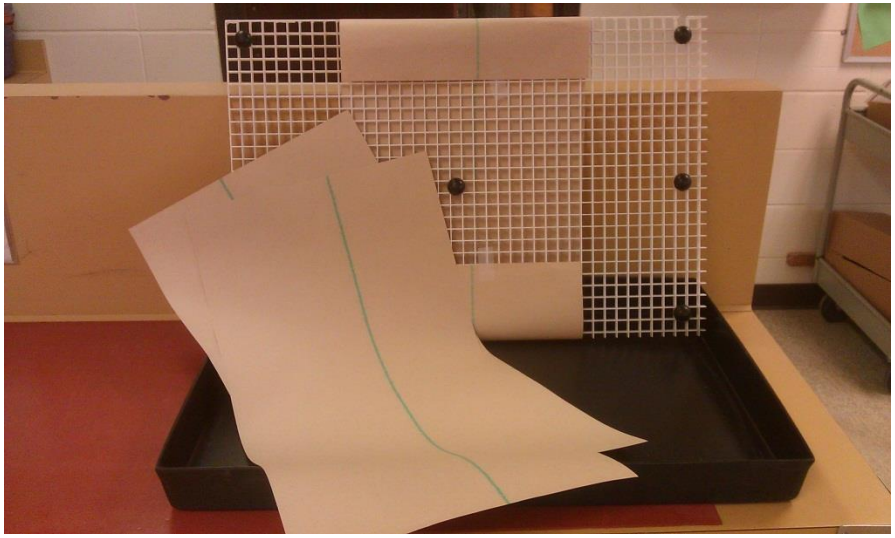
<u>Preparation</u>

- Paper towel (30.5 cm X 61.0 cm) is wrapped "sideways" on egg-crate with ends hanging below. Egg crate is elevated by rubber stoppers.
- Egg crate is placed in a tray and two towels are placed on top. One liter of water is added to towel (excess goes below egg crate).
- Finely sifted soil is added, tray returned to cart in 10°C overnight.

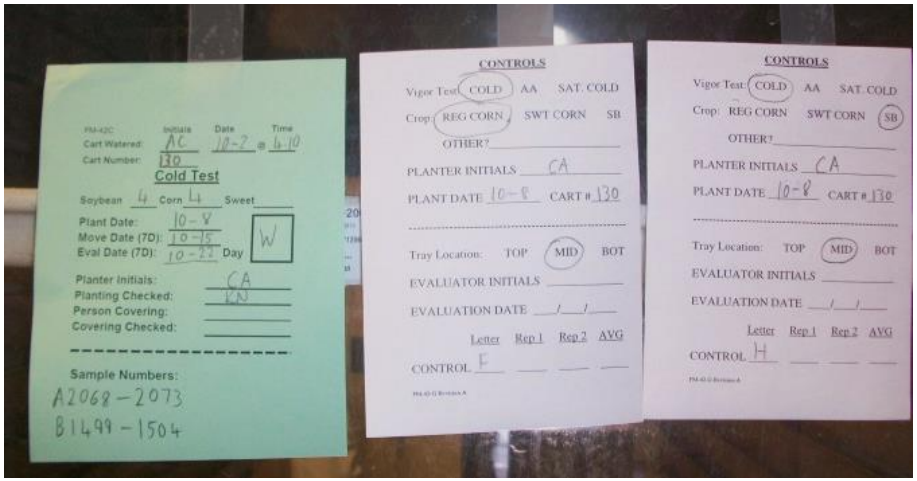


Saturated Cold Test

- Seeds are placed on soil. Corn – seeds flipped embryo-side down (flat) on soil.
- Cart is placed in 10°C for seven days without light.
- Samples are then placed in 25°C for 3-4 days.
- Evaluate according to AOSA Rules with allowances for early growth stage of seedlings.



Controls (Check Samples)



- Freeze seeds to maintain vigor level. Next best option is to store in cold room (10°C, 5°C, etc.).
- Options:
  - Run one check sample with border-line vigor (mid-80?)
  - Run two samples (one with vigor in 90's, one with vigor in 80's) with each cart or batch of cold tests.
- Results of controls should be within two standard deviations of known mean.
- Do results outside two SD's mean certain retesting?

Evaluation of Cold Tests

- Classifying seedlings in cold tests is based on the appropriate Rules (AOSA, ISTA, M &P). However since cold tests and other vigor tests are not used as labeling information there is the option of tweaking seedling descriptions.
- The tweaking is to reflect the needs of a vigor test, such as estimating emergence under a wide range of growing conditions.
- Cold tests that typically have a shorter warm period (such as the saturated cold test or seven day cold test) tend to focus on finding characteristics that might be allowed in a test with a longer warm period (minor damage to the coleoptile tip, length of shoot or roots).
- On tests where there is a covering material seedlings are typically tugged on to determine there are adequate roots (tray method). Normal seedlings are counted (forward counting)
- On tests where the material is under the seedlings they are counted in place or removed as they are counted (saturated, rolled towel). Forward or back (counting abnormal seedlings and dead seeds) counting may be used.

Vigor Testing Tolerances

- Found in AOSA Vigor Testing Handbook
- Not available for all methods
- Mandatory retest?
  - Yes because available tables contribute to uniformity in testing.
  - No, because vigor tests aren't official methods (in the AOSA Rules and used for labeling).
  - No if using rep to rep tolerance table in section 13 because allowable spread is too tight for vigor testing(?)

Cold Test Tolerance Table

Table 4.14 Maximum tolerance values for comparing two 200-seed cold tests of the same or different submitted samples tested in the same or different laboratories (2-way test at p = 5%).

Average Percent germination		Tolerance	Average Percent germination		Tolerance
A	B	C	A	B	C
99	3	2	74	27	15
98	5	3	73	28	15
97	5	4	72	29	15
96	6	5	71	30	16
95	7	6	70	31	16
94	7	7	69	32	16
93	8	8	68	33	16
92	9	9	67	34	17
91	9	10	66	35	17
90	10	11	65	36	17
89	10	12	64	37	17
88	10	13	63	38	17
87	11	14	62	39	18
86	11	15	61	40	18
85	12	16	60	41	18

Tolerance table for cold test reps?

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Germination

6.6a. Retest when difference between high and low reps exceed the maximum tolerated range (table 14I)

6.6e. No two satisfactory tests are within tolerance (Table 14J)

What do the results mean?

Cold Tests

Tray cold test	Cut-off 80%? 82%?
Saturated cold test	Cut-off 80%?

How was the saturated cold test conducted?

- Soil ¼" thick? Just enough soil to cover paper towels?
- How many days at 25°C?

- Is the Vigor Testing Handbook only a guideline?

Cold Tests and Field Emergence

- Cold test results have been correlated with field emergence. Although it is unrealistic to expect a consistent relationship over a wide range of differing conditions

  Which field conditions are we trying to predict?

  Mildly unfavorable or severe?

  Or something in between?
- Numerous experiments have repeatedly demonstrated the close association of cold test results with field emergence of corn as well as other crops.
- The inability of the cold test to accurately measure seed vigor of many crops can perhaps be attributed to the use of inappropriate temperature regimes.
- Therefore … making the cold test more widely applicable to many crops is establishing specific temperature regimes for specific crops and varieties.

SVTH

Why do results vary for the same method?

- P. 65: "The major sources contributing to variation, and therefore impeding standardization, are methodology and soil moisture".
- Differences in classifying seedlings
- Tweaking of methods
  - Temperature seeds and substrata are kept during planting, etc.
  - Temperature during cold period
  - Depth of soil, orientation of seeds (saturated cold test)
  - Soil (type & presence of pythium), planting media
  - ?????
- Makeup of samples (border-line seedlings)
- Uniformity of samples, Sampling

Importance of Sampling

- Test results only reflect the quality of the sample being tested.
- For those results to accurately reflect the quality of the whole seed lot, the examined sample should be a true representative of the seed lot.

SVTH, Page 26 V. Proper Sampling Techniques

Vigor Testing Publications

- AOSA Seed Vigor Testing Handbook
- ISTA Handbook of Vigour Test Methods
- Understanding Seed Vigor (pamphlet)
- SCST Seed Technologist Training Manual
- Principles of Seed Science and Technology
  Copeland & McDonald
- Seed Testing Principles & Practices.
  Elias, Copeland, McDonald, Baalbaki
- Ohio State University DVD. McDonald

AOSA Seed Vigor Testing Handbook

Major Revision in 2009

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Questions?

Comments?

Thank you!