Developing Herbicide BioAssays

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HERBICIDE BIOASSAY

• Used to determine presence or absence of specific herbicide trait

• With statistics & a good sample, gives estimate of lot purity

• Typically not for establishing field efficacy levels or compatibilities

• Not always suitable for event confirmation by itself.
HERBICIDE TRAITS

• Highly visible trait that is important to growers, need accurate test and confidence in results

• Events may be detectable by other methods, but herbicide bioassay shows trait is expressed, not just present
TRAITS

• Single Trait/event

• Stacked Traits
  • 2 or more traits that segregate independently
  • Need to be tested individually
    • Bt11 plus CRW604 plus GA21 = 3 events stacked together

• GM Linked Traits (Molecular stacks)
  • Created as one insert/event
  • Present as one genetic unit (won’t have one without the other)
    • Bt11 – Liberty linked to Cry1Ab, HX1 – Cry1F linked to Liberty
  • May need to verify both, test one at higher level
ASSIGNMENT

Develop a method for testing purity of seed lots of crop “X” bearing herbicide trait “Y”.

Where to start?
NEED

✓ Understand Purpose
✓ Select Method
✓ Knowledge of Species
✓ Understanding of Herbicide
✓ Knowledge of Trait / Events
✓ Validation Plan
✓ Ability to score and report
✓ Statistics
The bioassay test is used to determine what percentage of the viable seeds in the seedlot express the herbicide trait or event.

The bioassay should represent what a grower will see in his/her field when herbicide is applied to the seed lot.
SELECT TEST METHOD

- Lab Standard? – modifications?
- Other established method?
- Unique or new method?
- Contract obligations?
- Resources? - check SCST web site, peers...
- Typically use in-house standard method, if appropriate
COMMON TEST METHODS

- Substrate Imbibition
  - Rolled Towel
  - Kim Pac
  - Blotter boxes
- Seed Soak
- Spray
Find ideal conditions for species growth and normal development

• Corn – tolerant to moisture & temps
• Soybean – wet feet can be deadly
• Canola – happy to be cool
• Cotton – warm and fast

Need healthy fast growing plants to be able to select for herbicide effect
UNDERSTAND SPECIES

• Test species with chosen method without herbicide
  • Find ideal conditions
  • Detect abnormalities

• Hybrid or Variety, Inbreds also?

• Obtain pure, trait positive seed to develop test

• Seed to test - Good quality: variety, inbred, research, seed quality issues need to be addressed and equivalent controls/checks used
UNDERSTAND HERBICIDE

• SAFETY – MSDS
• Mode of action
• Rates of application
• Surfactants
• Formulation
  • i.e. Glyphosate 17%, 41%, 48%...
UNDERSTAND TRAIT

- Different events *may respond* differently to a herbicide
  - LL Corn - Bt11, LL (T25), HX1 – test strips
  - RR Corn – GA21 & NK603 - test strips
  - RR Soybeans, sugarbeets etc.

- New events & species all the time

- Linked vs Stacked Traits

- Follow up options available – test strips, PCR
VALIDATION PLAN

• Once parameters are set, validate

  • Use spiked samples in a blind test to verify readings are accurate and repeatable

    • Spike Seed should be of similar quality and treatment to expected samples

    • Analyst reading test should have no knowledge of actual spike levels

    • Validation tests must follow protocol for testing

• Set requirements for spike test results to pass or fail

• VALIDATE each time a protocol is significantly changed
DESIGNING THE TEST

• Start with selected test type

  • Set up a systematic Rate Series or Matrix of treatments
    • Include +/--checks
    • Include water checks (to show how plants grow in test conditions when not exposed to herbicide)

• Goal of Bioassay: Trait positives are healthy, negatives are symptomatic & abnormals are detected.
TEST DESIGN

- Bracket expected rate. Predict 800ppm?
  - 600 ppm
  - 800 ppm
  - 1000 ppm etc.

- Test positives & negatives *in each treatment*

- Use 3 + reps per treatment, if reasonable (if very unsure of range, may do less reps/seeds and more rates initially)

- Probably start with one formula

- Use water controls – verify plant growth

- Test appropriate materials – hybrids & inbreds
NEXT STEP

• If find no good treatment
  • Shift ppm scale or other parameters
  • Was non-treatment growth good?
  • Verify herbicide is correct for that event

• If obtained a good treatment
  • Repeat test (or treatment) to verify
  • Or Optimize
  • Use “Spiked” samples
  • Blind reads

• Document  - Write Protocol
A GOOD TEST IS

- **Validated** – to be appropriate and reliable. Gets correct result
- **Repeatable** – equal results each time
- **Robust** – critical range of variation tolerated
- **Rugged** – reproducible by other labs or staff
- **Accurate** – appropriate, low error rate. ID normals/abnormals, trait positives & negatives.
- **Documented** – Has correct, logical protocol
- **Only as good as the sample**
An appropriate, fit for purpose, bioassay must be able to distinguish and be able to distinguish:

- Abnormals from Normals
- Viable from non-viable
- Trait positives from Trait Negatives

Determine reporting requirements

- Report needed may affect sample size to test
- SAMPLE can be 100% positive, use straight math
- SEEDLOT is not reported as 100% pure unless all seeds are tested can report % purity of seed lot with a confidence level
Sample purity is reported as % normal tolerant compared to % non-tolerant in test.

Seedlot results may be reported based on confidence level.

- 95% is common choice.
- A retest is 95% likely to give a result within the same range (assuming good sample & test).

For sample with 4 non-tolerants and 396 tolerants:

- Sample = 99% pure.
- Seedlot report can by 95% confidence of >97.7%.
### AT 95% CONFIDENCE

<table>
<thead>
<tr>
<th>#K Tested</th>
<th>Stated Sample Purity</th>
<th>Upper Limit of lot</th>
<th>Lower Limit of lot</th>
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<tr>
<td>100</td>
<td>97%</td>
<td>99.4</td>
<td>91.5</td>
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<tr>
<td>10000</td>
<td>97%</td>
<td>97.3</td>
<td>96.7</td>
</tr>
</tbody>
</table>

Based on Seed calc program
ROUTINE TEST VALIDATION

• Used to monitor test and analysts

• In-House Validation
  • Regularly scheduled or Random often by percentage of volume
  • Typically uses spiked samples, blind
  • Test all analysts or technicians
  • Tests ruggedness & accuracy
  • Perform as if normal test

• Validation or Ring Tests
  • ISTA, USDA, SCST...
  • Trait Provider
QUESTIONS?