Determination of Tank-Mixture Efficacy

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Agenda

• Colby’s Analysis – a look into herbicide interactions, etc.
• Flint’s analysis – based on Colby’s interaction model
• The experiment: best practices...practical methods
• Questions & Discussion

The Colby Analysis

• Based upon an ‘expected’ level of control from mixing two or more herbicides together.
• Arguably one of the most cited papers in weed science.
• The benchmark for an enormous amount of intellectual property
The Colby Analysis

• The equation: \( E = \frac{X \times Y}{100} \), where....
  - X and Y are the effects of the herbicides applied alone (expressed as percent-of-control),
  - So, simplistically, if \( X = 50\% \) and \( Y = 40\% \) of control...
  - Then, \( E = \frac{(50 \times 40)}{100} \), or \( 20\% \) of control....

• The actual value for the herbicide mixture is then compared to the 'expected' value, and..
  - If greater = synergism
  - If less than = antagonism
  - If equal = additive

The Colby Analysis

• The advantages
  - Simple and straightforward
  - The data used in the analysis can be anything: visual observations, dry/fresh weights, weed counts, etc
  - It remains the benchmark method for measuring herbicide interactions

The Colby Analysis

• The disadvantages
  - Can be confusing:
    • different outcomes if different measures are used (for example, fresh versus dry weight)
    • Different outcomes from different mixture ratios...some rate combinations may be additive, some synergistic, some antagonistic
    • Mixtures identified as synergistic or antagonistic may not be statistically different from the herbicides used alone. (this is what Flint's analysis tests, and if it's not statistically different, it would be considered an additive mixture)
  - No adequate statistical companion (connection to Flint's analysis – or Chi square as suggested by Colby)
The Colby Analysis

• Just what was Bob thinking when he set this ‘expectation’?
• What are some of the concerns when determining the titration for interaction experiments?

Flints adaption to Colby’s analysis

• How would you statistically test for herbicide interactions with true physiological relevance?
• Flint’s interaction analysis is a statistical treatment of Colby’s Method
  – A modified analysis of variance (ANOVA) method for log-transformed data
  – Written for SAS

Flint’s adaptation to Colby’s analysis

Flint’s adaptation uses the vertical distance between the two log transformed biomass measurements to determine statistical parallelism
Parallel = Additive
Non-parallel = Synergistic/Antagonistic
Flint’s adaptation to Colby’s analysis

Additivity is EXPECTED = PARALLEL LINES

A mixture providing significantly LESS control than expected is ANTAGONISTIC

A mixture providing significantly MORE control than expected is SYNERGISTIC

Limitations
Too high of rates = non biologically-achievable expected values

Plant Biomass cannot have a negative value as seen here

This results in calculations of “False Antagonism” at high rate combinations (see Hugie et al. 2008 for example)

Decoding the SAS Code: Data organization (Example: *.CSV)

<table>
<thead>
<tr>
<th>Block species</th>
<th>Herbicide A</th>
<th>Herbicide B</th>
<th>Biomass</th>
<th>percent control</th>
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</table>

Rate of herbicide expressed in g/ha
Biomass expressed in grams dry weight or % of control
Flint’s adaptation to Colby’s analysis

Decoding the SAS Code: Program

- Log transformation gives linearity to dose response data for slopes’ comparisons
- Order of terms is very important for comparisons – keep this consistent
- This particular program is set up to analyze one rate of herbicide B, and multiple rates of herbicide A (0, 4, 8, and 12g)
- Reversal of all logM and logA terms for multiple rates of herbicide B, and one of herbicide A

Decoding the SAS Code: Output

3 biological replicates
10 plants per treatment
Two rates (0g & 10g) of herbicide B (logM)
Four rates (0, 4, 8, 12g) of herbicide A

No interaction between biological replicates and effect of herbicide A
No interaction between biological replicates of effects of mixtures
Effect of rates of herbicide A is significant
Significant interaction of herbicide A and B overall
Interaction of herbicide A and B at specific rates
< 0.05 = significant interaction
Estimate = negative = synergism & gives magnitude of deviation of slopes from parallelism or additivity
Flint’s adaptation to Colby’s analysis

- The advantages
  - Statistical relevance is identified
  - A range or series of rate combinations may be tested for significant interactions simultaneously
- The disadvantages
  - Does response/titrations need to be appropriate for model
  - Are there other disadvantages that you see?

Methods/Best Practices

- Suggested number of reps....4 or more
  - Weed species
- Minimum titration number....4 or more
  - Each additional mixture requires each herbicide to also be applied alone at rates in mixture
- Data collection options
  - Quantitative vs Qualitative
- Eliminate as much variability in experimental conditions as possible

Questions/discussion

- Do the herbicides need to have different modes-of-action?
- How to account for surfactant/adjuvant effects?
- Is it possible to patent a mixture for both synergism and antagonism (safening)?
References