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

- Colby, S.R. Calculating synergistic and antagonistic responses of herbicide combinations. *Weeds* **15** (1967), pp. 20–22.
- 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 = (X*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 = (50*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

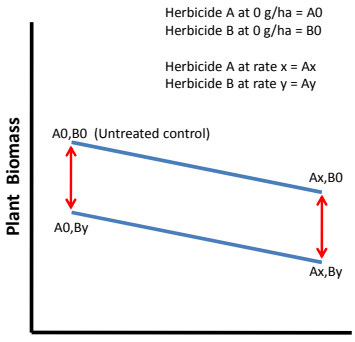
- How would you statistically test for herbicide interactions with true physiological relevance?
- Flint's interaction analysis is a statistical treatment of Colby's Method
  - A modified analysis of variance (ANOVA) method for log-transformed data
  - Written for SAS

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/Antogonistic

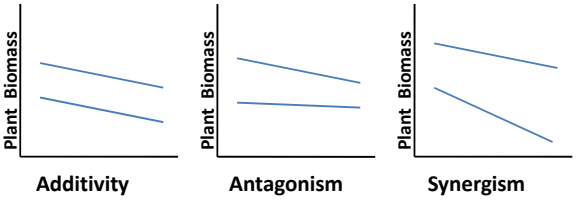


Flint's adaptation to Colby's analysis

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



Flint's adaptation to Colby's analysis

Limitations

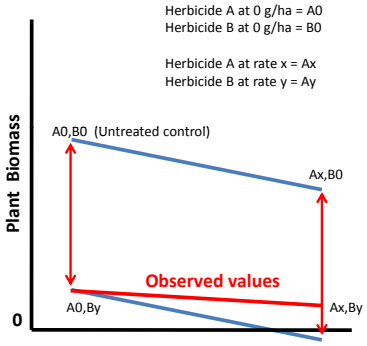
Too high of rates
= non biologically-achievable expected values

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)

Herbicide A at 0 g/ha = A0
Herbicide B at 0 g/ha = B0

Herbicide A at rate x = Ax
Herbicide B at rate y = Ay



Flint's adaptation to Colby's analysis

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

	A	B	C	D	E	F	G
1	studies	block	species	herbicideA	herbicideB	biomass	percentcontrol
42	1	1	RP	0	0	4.293	1.193627
43	1	2	RP	0	0	4.384	1.218929
44	1	3	RP	0	0	2.792	0.776289
45	1	4	RP	0	0	3.061	0.851082
46	1	5	RP	0	0	3.453	0.960073
47	1	1	RP	4	0	2.451	0.681477
48	1	2	RP	4	0	2.727	0.758216
49	1	3	RP	4	0	2.528	0.702886
50	1	4	RP	4	0	2.96	0.823
51	1	5	RP	4	0	2.586	0.719012

← Headers

Rate of herbicide expressed in g/ha

Biomass expressed grams dry weight or % of control

Flint's adaptation to Colby's analysis

Decoding the SAS Code: Program

```
/*Slopes Comparison: Adapted Colby's Method*/
/*Import data file, change filename as required*/
data synergism;
    infile 'C:\Documents and Settings\huggie\Desktop\SAS files\2004\test.csv' dlm=',' firstobs=2;
    input studies block species$ herbicideA herbicideB biomass percentcontrol;
      logB = log(biomass*1000);
    logM = log(herbicideB+1.75);
      logA = log(herbicideA +1.75);
      run;
data synergism;set synergism;
where species='RP';/*change biotype name*/
run;
proc mixed data=synergism;
      class studies block logM logA species;
      model logB = logA logM logM*logA/*The model is adapted Colby's method, the log transformation of the multiplicative model*/
      random studies*logA studies*logM studies*logA*logM;
      /*contrast statements comparing slopes on different sets of estimates*/
      estimate 'slope1' logM*logA  1 -1 0 0 /*0 0 0 0 0 0*/
                                  -1 1 0 0 /*0 0 0 0 0 0*/;
      estimate 'slope2' logM*logA 1 0 -1 0 /*0 0 0 0 0 0*/
                                  -1 0 1 0 /*0 0 0 0 0 0*/;
      estimate 'slope3' logM*logA 1 0 0 -1 /*0 0 0 0 0 0*/
                                  -1 0 0 1 /*0 0 0 0 0 0*/;
      /*estimate 'slope4' logM*logA 1 0 0 0 -1 0 0 0 0 0
                                  -1 0 0 0 1 0 0 0 0 0*/
```

"logM" = herbicide B
"logA" = herbicide A

- 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

Flint's adaptation to Colby's analysis

Decoding the SAS Code: Output

```
Data Set                        WORK.SYNERGISM
Dependent Variable              logB
Covariance Structure            Variance Components
Estimation Method               REML
Residual Variance Method        Profile
Fixed Effects SE Method         Model-Based
Degrees of Freedom Method       Containment

Class Level Information

Class      Levels  Values

studies         3  1 2 3
block          10  1 2 3 4 5 6 7 8 9 10
logM            2  0.5596157879 2.463853240
logA            4  0.5596157879 1.749199854 2.2772367285 2.6210588241
species         1  RP

Dimensions

Covariance Parameters           4
Columns in X                   15
Columns in Z                   42
Subjects                        1
Max Obs Per Subject           200
```

3 biological replicates

10 plants per treatment

Two rates (0g & 10g) of herbicide B (logM)

Four rates (0, 4, 8, 12g) of hebicide A

Flint's adaptation to Colby's analysis

Decoding the SAS Code: Output

```
Covariance Parameter Estimates

Cov Parm                Estimate

studies*logA                   0
studies*logM             0.1139
studies*logM*logA              0
Residual                 0.1152

Fit Statistics

-2 Res Log Likelihood          169.7
AIC (smaller is better)        173.7
AICC (smaller is better)       173.7
BIC (smaller is better)        174.7

Type 3 Tests of Fixed Effects

                Num    Den
Effect           DF     DF   F Value    Pr > F

logA              3      6     64.57    <.0001
logM              1      2      4.38    0.1715
logM*logA         3      6      4.93    0.0465

Estimates

                                Standard
Label      Estimate               Error     DF   t Value    Pr > |t|

slope1      -0.3606              0.1358      6     -2.66      0.0377
slope2      -0.3783              0.1358      6     -2.79      0.0317
slope3      -0.6917              0.1358      6     -5.62      0.0111
```

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

- Colby, S. R. 1967. Calculating Synergistic and Antagonistic Responses of Herbicide Combinations. Weeds. 15: 20-22.
- Flint, J. L., P. L. Cornelius, and M. Barrett. 1988. Analyzing herbicide interactions: a statistical treatment of Colby's method. Weed Technol. 2:304–309.
- Hugie, J. A., Bollero, G. A., Tranel, P. J., and Riechers, D. E. 2008. Defining the rate requirements for synergism between mesotrione and atrazine in redroot pigweed (*Amaranthus retroflexus*). Weed Sci. 56:265-270.