BETTER DESIGN AND ANALYSIS FOR LONG-TERM EXPERIMENTS

Thomas M. Loughin

Department of Statistics

Kansas State University

loughin@stat.ksu.edu

The ASA-CSSA-SSSA International Annual Meetings

November 6-10, 2005 Salt Lake City, UT

BETTER DESIGN AND ANALYSIS FOR LONG-TERM EXPERIMENTS

Collaborators:

Mollie Poehlman Roediger
University of Minnesota

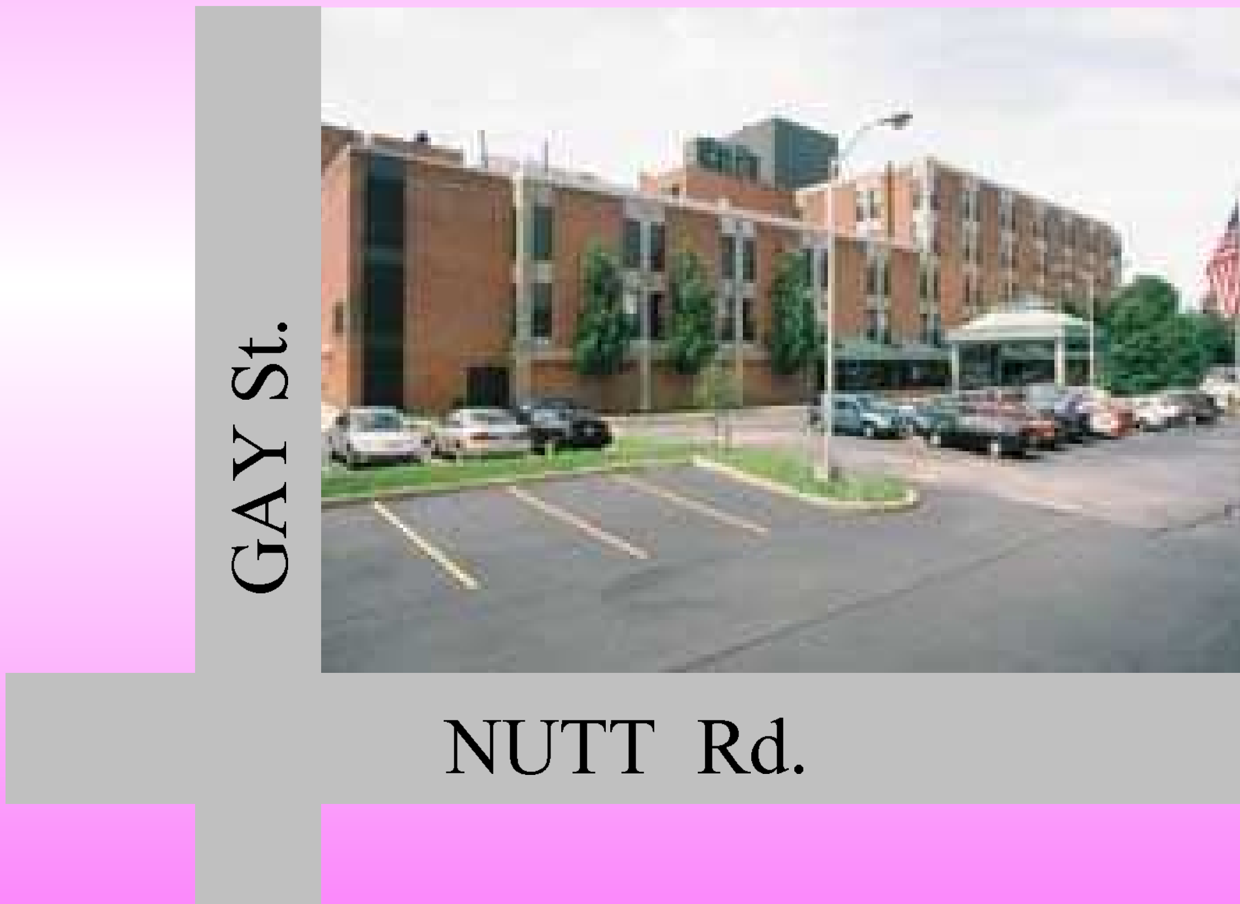
George A. Milliken
Kansas State University

John P. Schmidt
USDA, State College, PA

Slides from talk available at
`www-personal.ksu.edu/~loughin/STATPAGE.html`

Who am I ?

I was born here:



Who am I ?

- MS Stat 1987 (UNC-CH)
- 2 Years at Quintiles (Stat consulting for Pharmaceutical Companies)
- PhD Stat 1993 (Iowa St.)
  - 4 years in Ag Experiment Station Stat Consulting Center
- At Kansas State since 1993
  - 12-month appointment, including 50% funding from K-State Research and Extension (one of 6 faculty consultants)

Why am I here?

- Spent one afternoon/week for 12 years in Plant Sciences Center
- Have helped to design and analyze hundreds of experiments in plant sciences
- Have handled dozens of problems on Long-Term Experiments
  - Have never been satisfied with my own advice
  - They *look* like ordinary repeated-measures problems
  - They are *fundamentally different* from ordinary repeated measures problems.
- Began researching the problem...

Goals of this talk

1. Review "standard practice" in analysis of long-term experiments
2. Explain why none of these methods are correct for Long-Term Experiments
3. Offer suggestions for improving analysis
   - Explain why nothing *can* work perfectly
4. Place blame on the *design* of the experiment
5. Offer suggestions for improving design of long-term experiments

Familiarity with general ANOVA concepts is assumed.

What is a LTE?

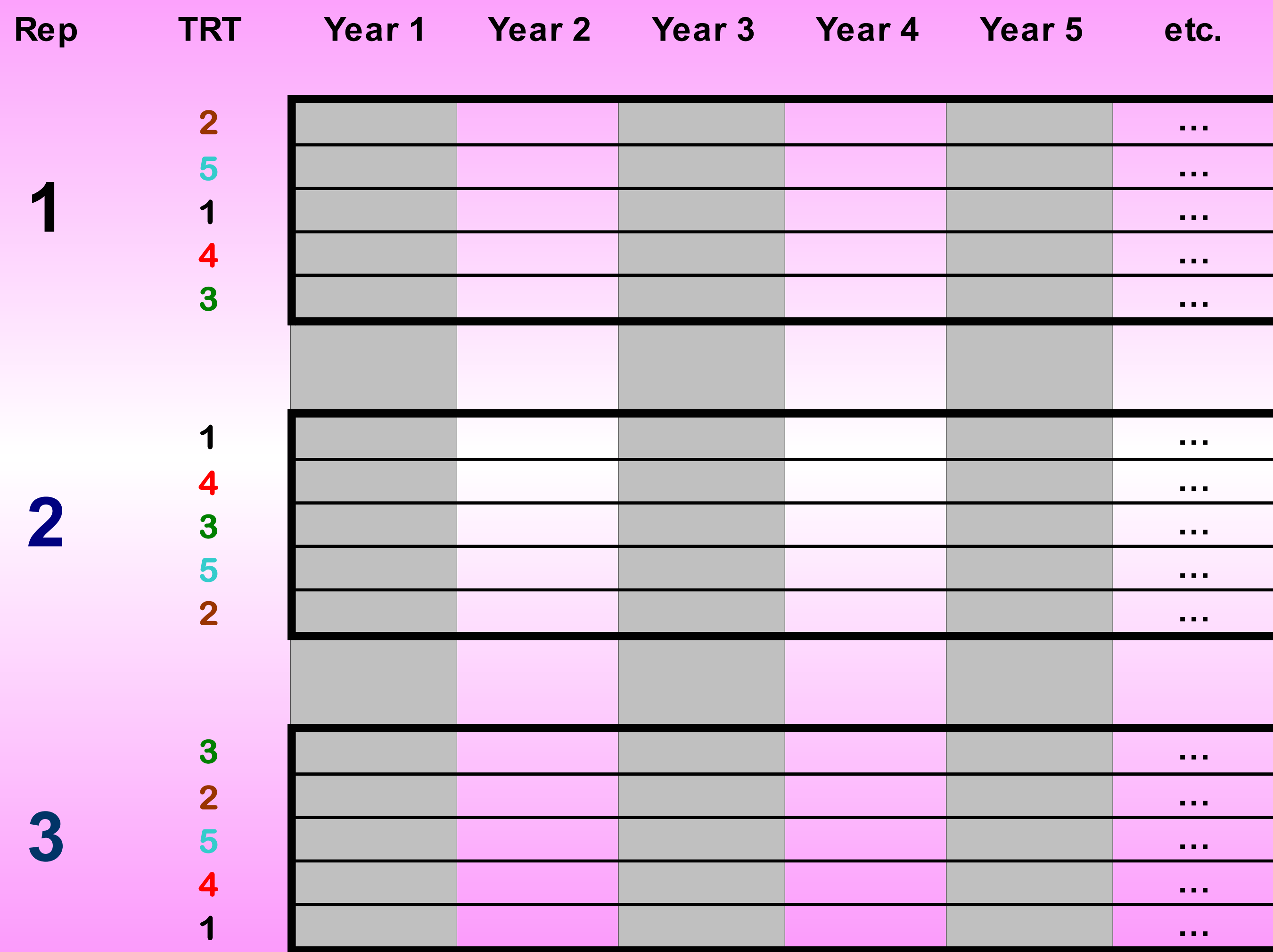
Long-Term Experiments (LTEs) are

- Conducted to compare long-term effects (e.g. sustainability) of various "Treatments" (TRTs) on some "response(s)" (Y):
  - Soil Fertility and other properties
  - Erosion
  - Crop Yields
  - Pest Control
- "Long" term:
  - Many years (Rothamsted "classicals" $>$ 100 years)
  - A few years ("Young", or with shorter-term goals)

Typical Design of an LTE

- Select an experimental design (commonly RCB)
  - Additional features, such as factorial treatment structure or split-plot design, may be used as needed
- Assign treatments (TRTs) to units (plots)
  - TRTs may be one-time applications, continual or varying practices
  - Tillage, chemical applications, rotations, etc.
- TRTs assigned at start of experiment never change
- *Measure response on each unit (plot) repeatedly over time*
  - Annual (crop yield) or more often (soil/plant properties)

Schematic of a Basic LTE

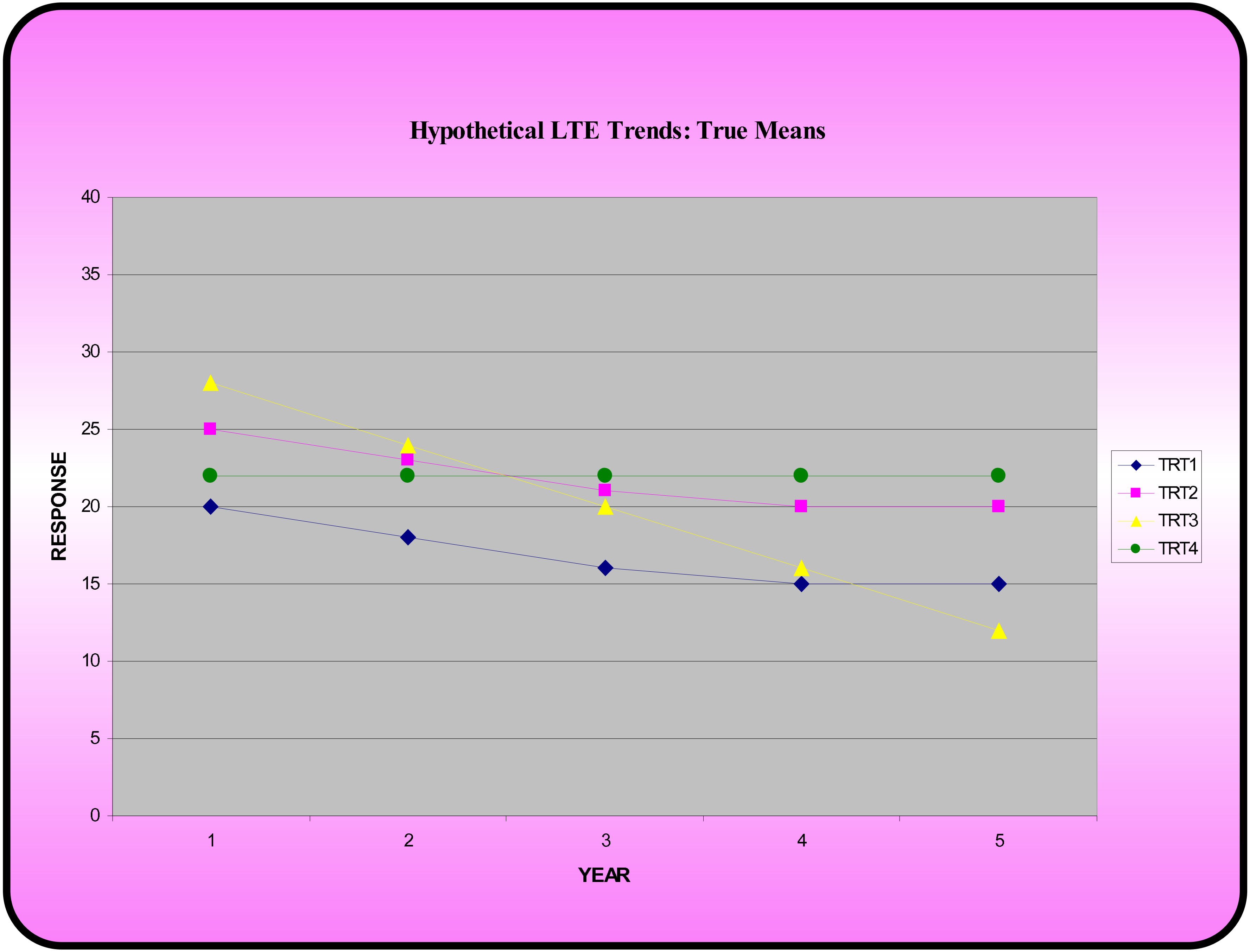


Analysis goals of a LTE

Primarily:

1. Understand differences in TRT effects over time
2. Understand how means change over time for different TRTs

These are *Interaction* effects between Time and TRT


Hypothetical LTE Trends: True Means
40
35
30
25
20
15
10
5
0
RESPONSE
1
2
3
4
5
YEAR
TRT1
TRT2
TRT3
TRT4

Analysis goals of a LTE, cont'd

Secondarily:

1. Determine whether there is an overall time effect on the measurements
2. Whether there are overall TRT effects on the measurements

These are *Main Effects* of time and TRT, respectively.

Data from a LTE

Special kind of repeated measures:

- Multiple measurements made over time on each experimental unit
  - Often annual, but can be any intervals
  - Often equally-spaced in time, but not necessarily
- *Measurements taken on all units at the same time*

Repeated Measures Analysis

Three approaches used in most cases:

1. Derived Variables
2. Multivariate Analysis
3. Modeling of Serial Correlation
   (a) Split Plot (assuming correlation is constant)
   (b) Other models (allowing correlation to vary)

Derived Variables Approach

General idea:

1. Reduce the multiple measurements on each unit to one or more "composite" variables that measure some phenomenon of interest. Some examples:
   - Total (or average) response over whole experiment
   - Total (or average) response over some special interval
   - Response at a particular time
   - Slope of response over experiment or special interval
   - Change in response between two chosen points
   - Maximum response across all times
   - Minimum response across all times

Derived Variables Approach, cont'd

1. In each case, the variable is computed separately *for each unit in the study.*

2. Analyze values of the derived variable according to design of experiment

   - ANOVA for RCB, or whatever design is
   - Contrasts or pairwise comparisons among TRTs

Derived Variables Approach, cont'd

Advantages:

- Easy to do analysis
- Easy to interpret results

Disadvantages:

- No formal F-test of Time, TRT, Time*TRT
- Possibly very many tests if numerous different derived variables are considered
  - Inflated risk of Type I Error (false rejection)
- Reducing data to simpler forms may reduce power for some comparisons

Derived Variables Approach, cont'd

In my opinion this is a nice approach to repeated measures — you can test very concise questions just by defining appropriate derived variables and analyzing them in a relatively simple manner.

*Multivariate Analysis Approach

- Responses $Y_1, Y_2, \ldots, Y_r$ for measurements over r times
- Multivariate ANOVA (MANOVA): Simultaneously model $Y_1, Y_2, \ldots, Y_r$ according to design of experiment
  - Effects of TRTs are compared on "best" combinations of response variables as determined by correlation structure among them
  - A single test simultaneously compares TRTs at all times
  - Separate tests at each time are often done as a follow-up

*Multivariate Analysis Approach, cont'd

Advantages:

- Uses information contained in responses in an "optimal" way to best distinguish among TRTs
- Has good power in some circumstances

Disadvantages:

- No control over what combination of variables gets tested! (The data decides)
- Results can be difficult to interpret
- Has very low power if there are many times relative to degrees of freedom for error

*Multivariate Analysis Approach, cont'd

In my opinion this is not a very useful method of analysis for most repeated measures problems.

(It is not often used.)

Modeling the Serial Correlation

"Serial correlation" = correlation over time.

Cochran (1939) recognized that LTE data "looks like" data from a split plot:

Example of Measurements in a Split Plot

W.P. Unit 1	W.P. Unit 2	…	W.P. Unit n
W.P.TRT 3	W.P.TRT 1	…	W.P.TRT 2
S.P TRT 1, S.P TRT 3, S.P TRT 4, S.P TRT 2	S.P TRT 2, S.P TRT 4, S.P TRT 3, S.P TRT 1	…	S.P TRT 4, S.P TRT 3, S.P TRT 1, S.P TRT 2

Example of Measurements in Repeated Measures

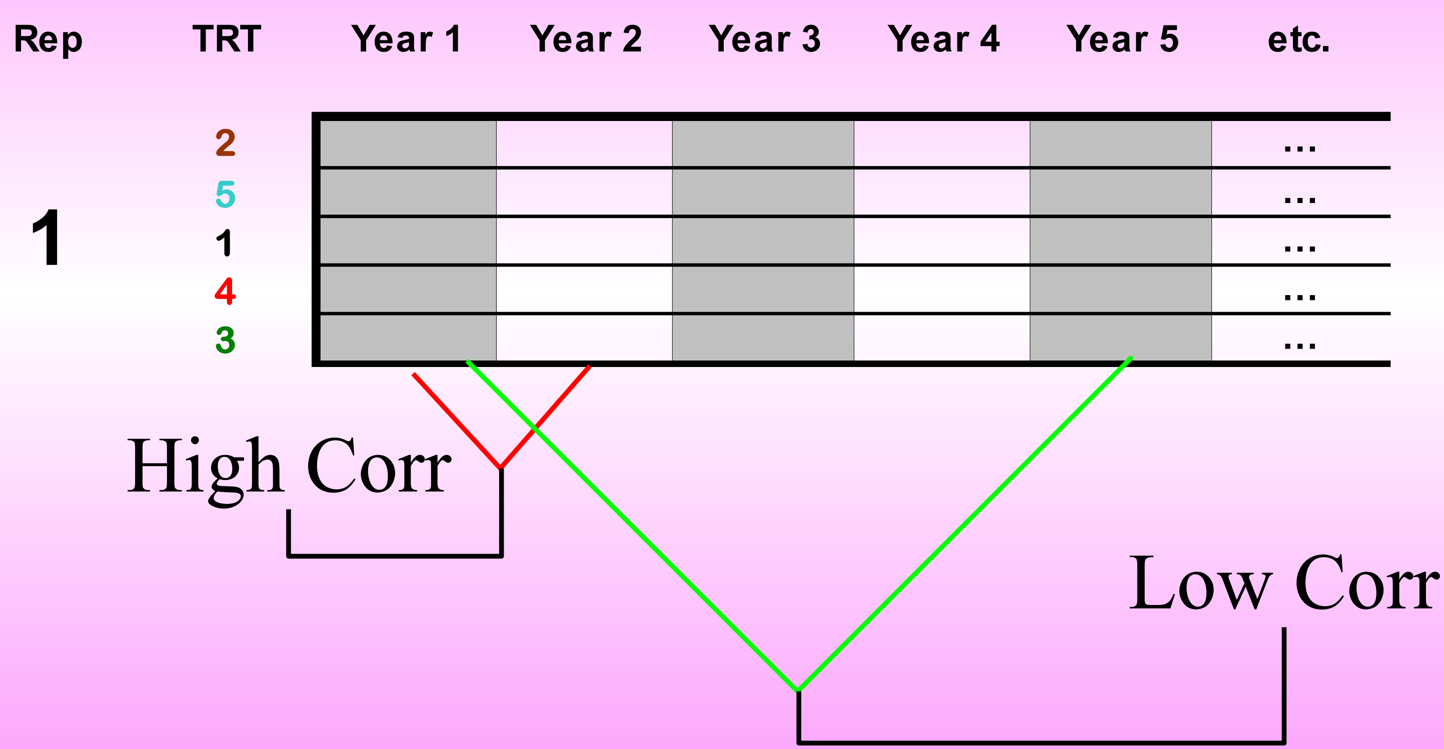
Unit 1	Unit 2	…	Unit n
TRT 3	TRT 1	…	TRT 2
Time 1, Time 2, Time 3, Time 4	Time 1, Time 2, Time 3, Time 4	…	Time 1, Time 2, Time 3, Time 4

Modeling the Serial Correlation, cont'd

Cochran (1939) also recognized that

- Measurements taken close together in time are often more closely related to each other than measurements taken farther apart.
- Therefore, serial correlation is higher for neighboring times than distant times.

Modeling the Serial Correlation, cont'd



Modeling the Serial Correlation, cont'd

Cochran (1939)

- Didn't have the tools to model the serial correlation, and
- Recognized that using a split plot wasn't really right

(This is what led him to the derived-variables regression approach.)

Nonetheless, split-plot analysis of repeated measures became popular!

This is potentially very bad, depending on correlations!

Modeling the Serial Correlation, cont'd

We now have tools to model serial correlations, so there is no longer any need to analyze repeated measures as split plots.

`PROC MIXED` in SAS can fit many different correlation models (So many, in fact, that now you need to know how to choose!)

```
repeated TIME / subject=_________ type=___;
```

Modeling the Serial Correlation, cont'd

"Modern" approach to repeated measures analysis:

1. Fit several different candidate correlation models to the data
2. Use some established criterion to select a model with the "best" fit
   - Guerin and Stroup (2000) and new Analysis of Messy Data: AIC
3. *Assume that this is the correct model* and use it to perform tests of Time, TRT, Time*TRT, and follow-ups

Modeling the Serial Correlation, cont'd

Advantages:

- "Thorough" analysis
  - Provides formal tests of Time, TRT, Time*TRT
  - All Time*TRT combinations can be compared in any way through selected contrasts
- Potential gain in power when the correlation model is chosen correctly
  - Comparisons at a given time are "augmented" by correlated results at other times.
- Appeals to people who like to use the "latest thing"

Modeling the Serial Correlation, cont'd

Disadvantages:

- Computationally intensive, especially with many times
  - Some models may fail to yield results
  - End up with "best of those that worked"
- Substantial chance of choosing wrong correlation structure
  - This can result in poor test results.
- Requires more assumptions than other methods
- Follow-ups often merely TRT comparisons at each time
- No real gain in power if heterogeneous variances across time

Modeling the Serial Correlation, cont'd

In my opinion this is a useful procedure in a limited number of problems

- When a formal test of Time*TRT is needed
- When an understanding of the serial correlation is desired
- When no derived variables are interesting

Interim Conclusion

There are several viable methods for analyzing repeated-measures data.

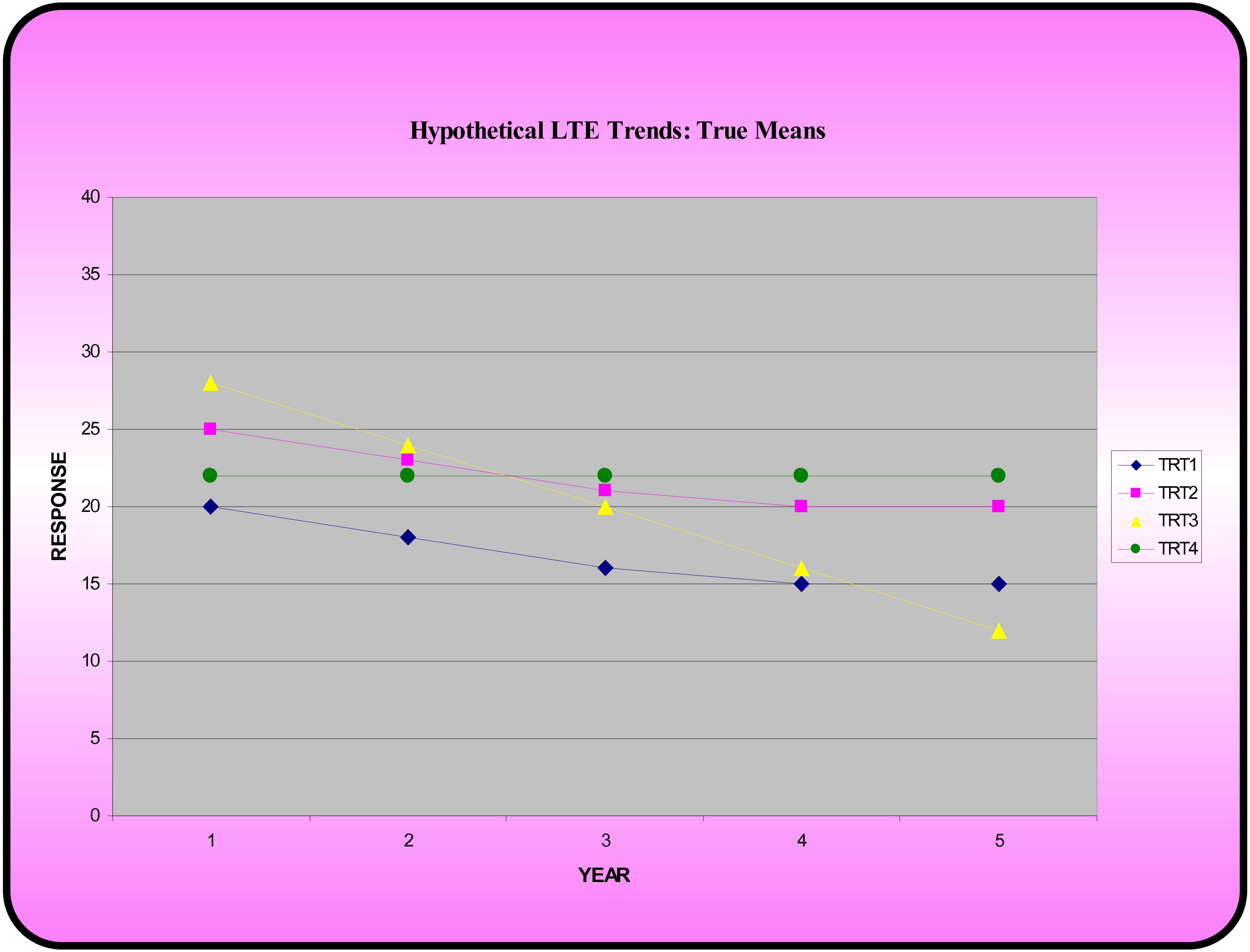
Each has benefits and drawbacks.

Each has been used in published analyses of LTEs

Each of these methods is wrong for LTEs!

Fundamental Problem with LTE Data

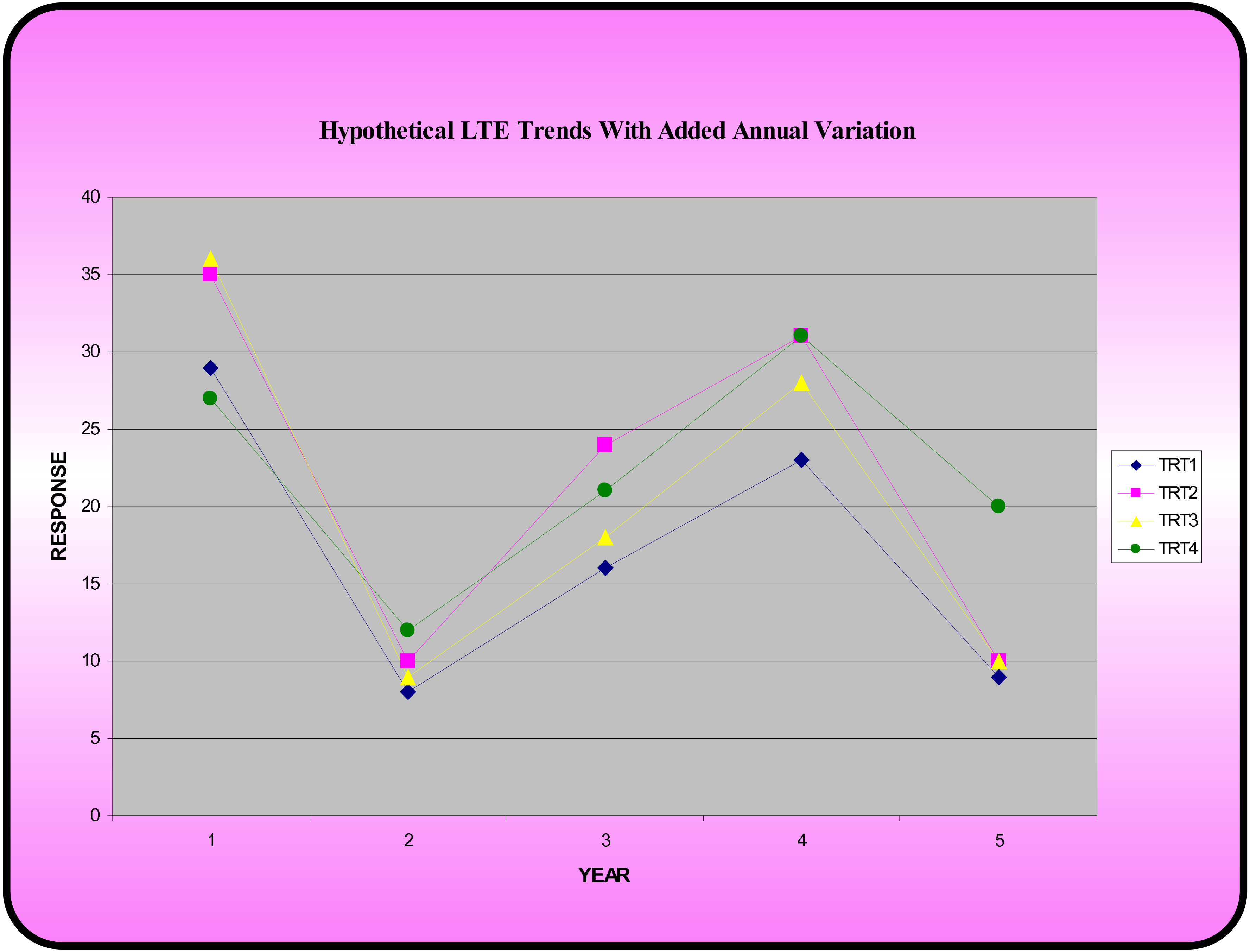
You are looking for this:



Hypothetical LTE Trends: True Means
RESPONSE
0
5
10
15
20
25
30
35
40
1
2
3
4
5
YEAR
TRT1
TRT2
TRT3
TRT4

Fundamental Problem with LTE Data

But what you have is this:


Hypothetical LTE Trends With Added Annual Variation
RESPONSE
40
35
30
25
20
15
10
5
0
1
2
3
4
5
YEAR
TRT1
TRT2
TRT3
TRT4

Fundamental Problem with LTE Data, cont'd

In an LTE, random annual variations in environments affect ALL units simultaneously.

- All units' responses rise and fall in unison
- Random interactions between TRTs and Environments further obscure treatment comparisons over time
- Violates primary assumption of all repeated measures analyses: **Units *must* respond independently of one another**
  - Often the most critical assumption in an analysis!

Fundamental Problem with LTE Data, cont'd

None of the standard repeated measures analyses can distinguish

- "Fixed" effects of time: repeatable, real trends
- "Random" effects of time: uncontrollable, unrepeatable fluctuations

Instead they combine the effects and test them simultaneously.

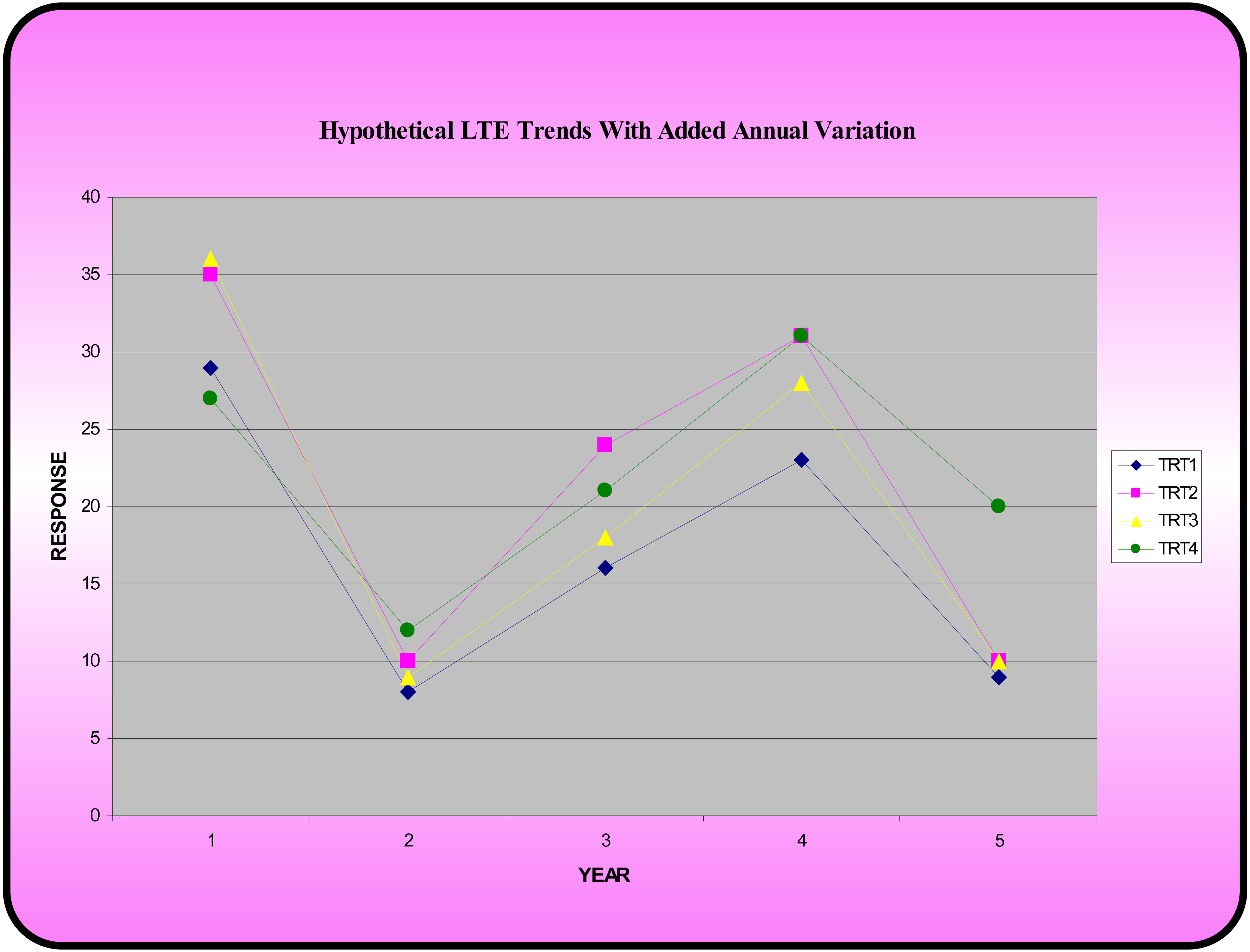
Analyses (Poehlman 2003, Bailey et al. 1996) show that random effects can overwhelm fixed effects.

- How do you interpret a significant F-test?

Can this be fixed?

No. $\ddot{\frown}$

- Flaw is in *design* of experiment
- Design **lacks replication** of year environments
  - Fixed and Random effects are "confounded" (inseparable)
  - No way to distinguish them without further assumptions
  - Conclusions limited to observed sequence of years!
    (Try predicting means at year 2!)


Hypothetical LTE Trends With Added Annual Variation
RESPONSE
YEAR
0
5
10
15
20
25
30
35
40
1
2
3
4
5
TRT1
TRT2
TRT3
TRT4

Improved Analysis of LTE data

By making *assumptions* about the form of certain aspects of the fixed trends or random effects, we can separate them.

For example, we can

1. Assume that the fixed trends follow a specific pattern
2. Assume that the random effects can be explained by other measurements
3. Assume that there is a specific amount of variability associated with the random effects.

Improved Analysis of LTE data, cont'd

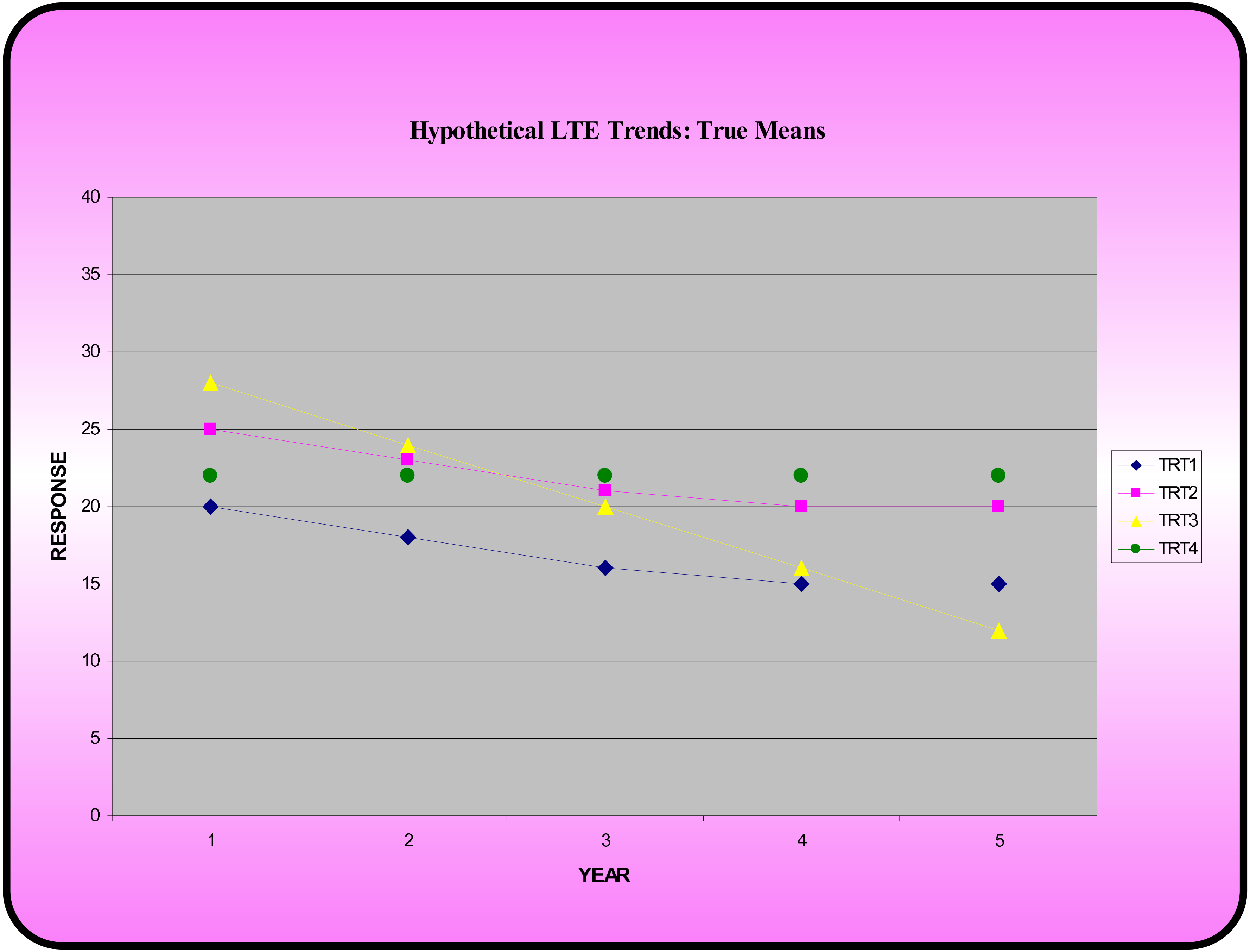
1. Assume that the fixed trends follow a specific pattern

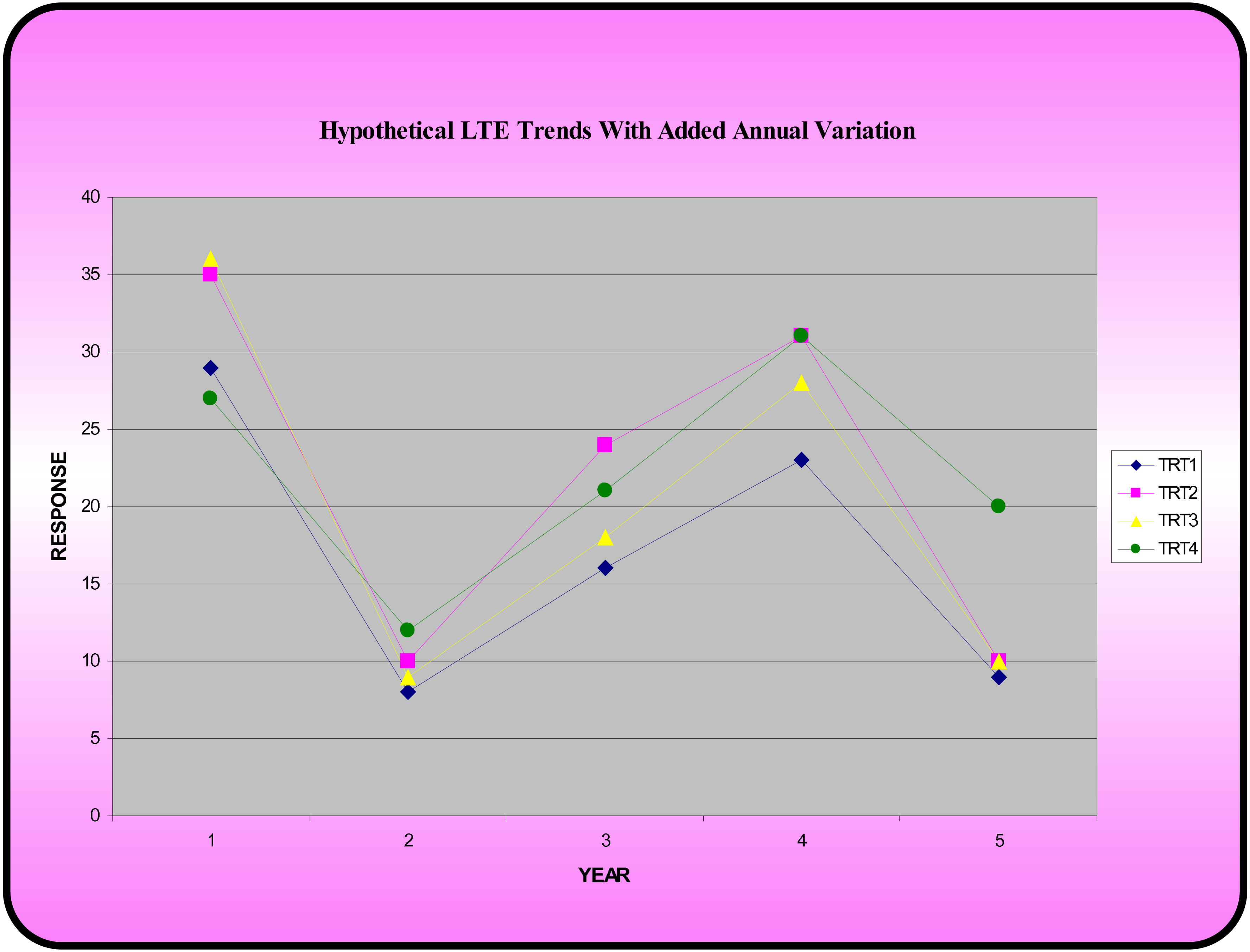
- *Assume* that the mean trends over time for each treatment follow some known equation
  - Linear regression
  - Polynomial regression
  - Exponential decay
  - Something else
- *Assume* that any deviation from the assumed model is due to randomness (and not failure of the model)
  - Use variation around fitted equation to estimate variance associated with random effects
- Incorporate this into method that models serial correlation

Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

Example: Let's see whether we can extract Truth from Data:


Hypothetical LTE Trends: True Means
40
35
30
25
20
15
10
5
0
RESPONSE
1
2
3
4
5
YEAR
TRT1
TRT2
TRT3
TRT4


Hypothetical LTE Trends With Added Annual Variation
RESPONSE
0
5
10
15
20
25
30
35
40
1
2
3
4
5
YEAR
TRT1
TRT2
TRT3
TRT4

Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

Example: Let's see whether we can extract Truth from Data:

Generated data from these latter means:

- Four reps
- Added a little bit of block variability
- Added a little bit of plot variability

Will assume linear trends for all treatments

*Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

ANOVA for this analysis (start with standard split plot):

Source	DF	
Block	$(b-1)$	$b = \#$ blocks (4 here)
TRT	$(t-1)$	$t = \#$ TRTs (4 here)
Block*TRT	$(b-1)(t-1)$	
Year	$(r-1)$	$r = \#$ times (5 here)
Year*TRT	$(r-1)(t-1)$	
Error	$t(b-1)(r-1)$	
Total	$btr-1$	

*Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

Add assumed time pattern

Source	DF	
Block	$(b-1)$	"Time" = numerical variable
TRT	$(t-1)$	representing fixed time levels
Block*TRT	$(b-1)(t-1)$	for linear trend
Time	1	
Year	$\boldsymbol{(r-2)}$	"Year" = Class variable
Time*TRT	$(t-1)$	representing random effects
Year*TRT	$\boldsymbol{(r-2)(t-1)}$	of environments
Error	$t(b-1)(r-1)$	
Total	$btr-1$	

*Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

`Proc Mixed` code for this analysis:

```
proc mixed method=type1;
  class block trt year;
  model y = trt time time*trt / ddfm=kr;
  random block block*trt year year*trt ;
  * Repeated time / type=... ; <--(not needed in example:
  lsmeans trt / diff at time=1;  no serial corr was added)
  lsmeans trt / diff at time=2;
  lsmeans trt / diff at time=3;
  lsmeans trt / diff at time=4;
  lsmeans trt / diff at time=5;
run;
```

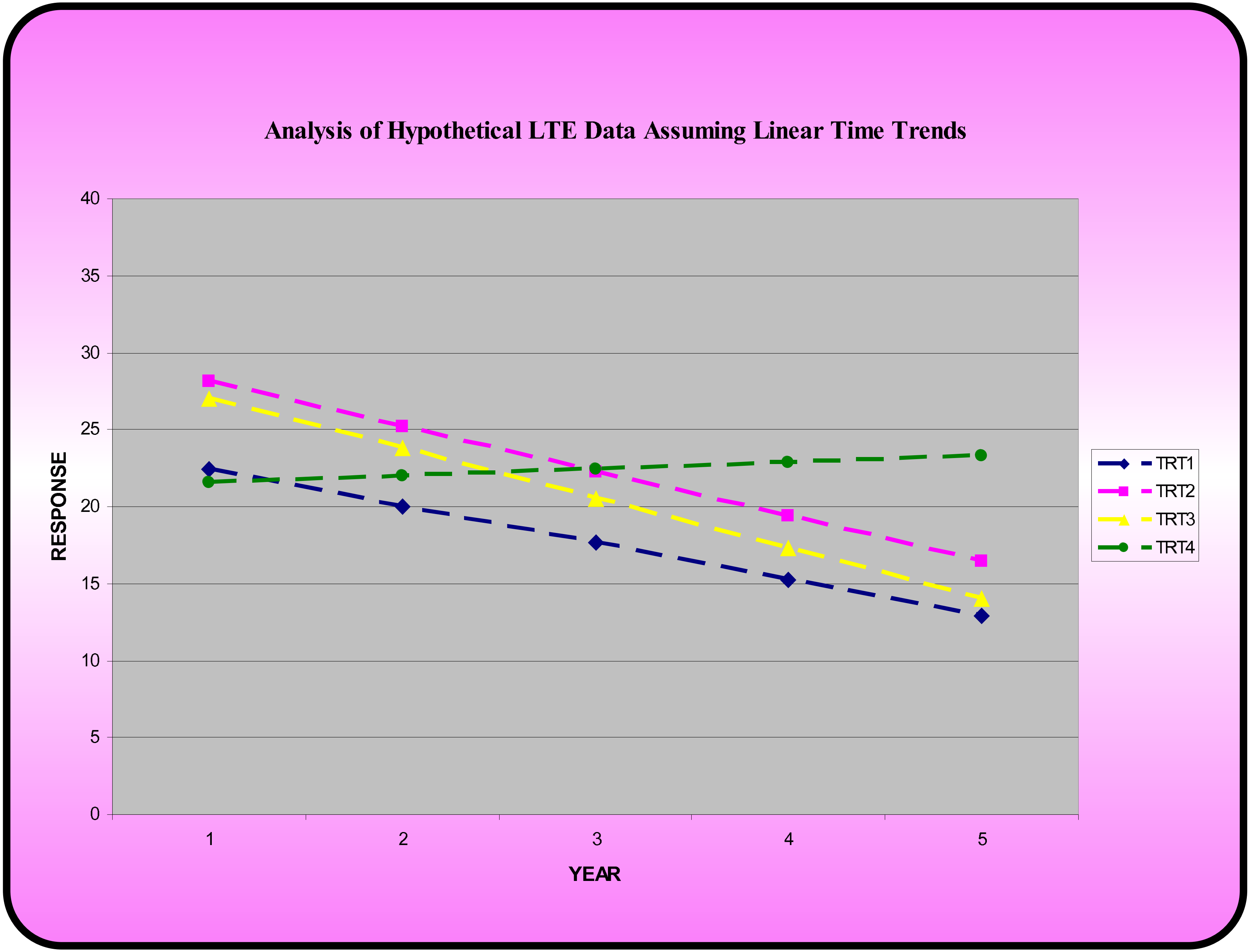
Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

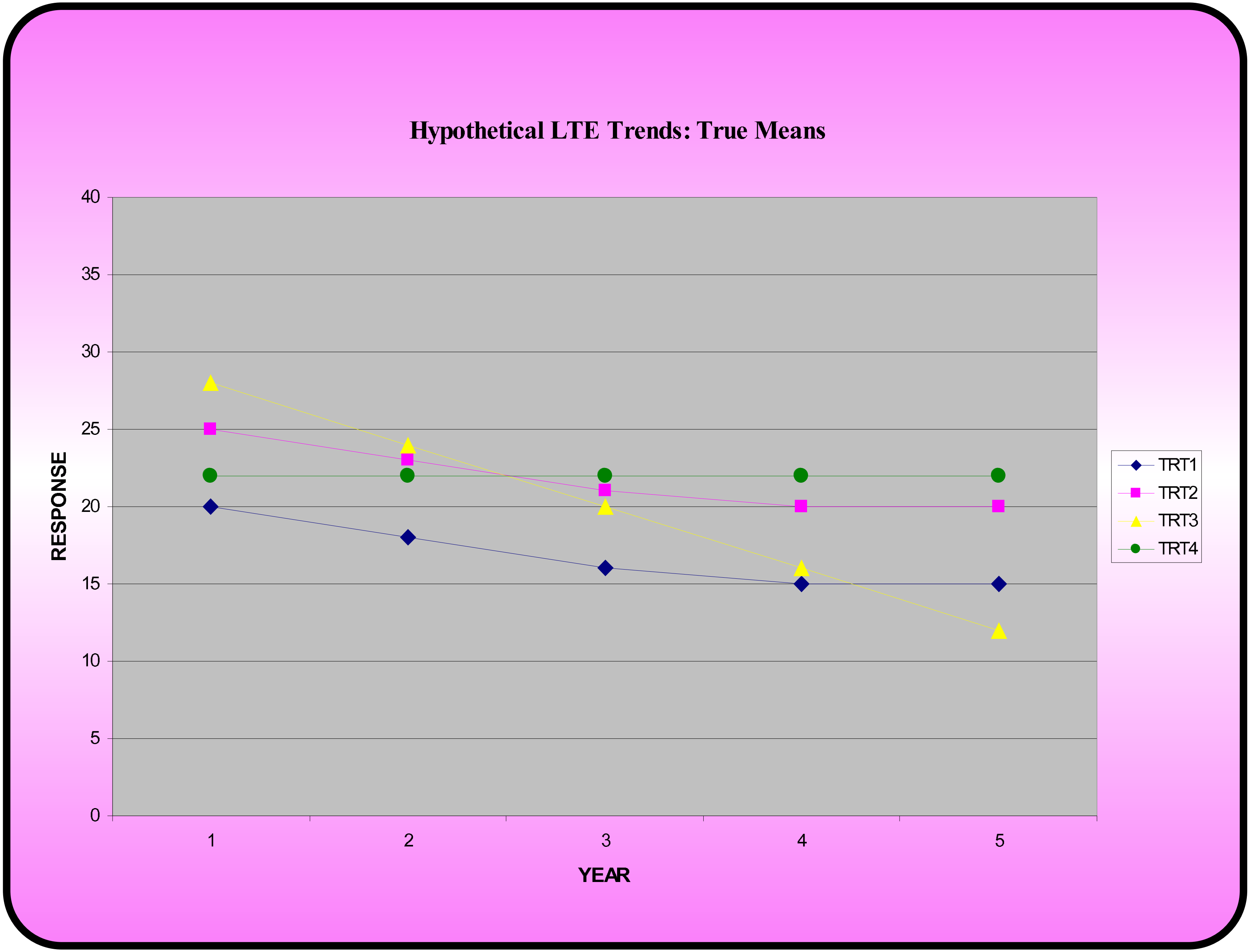
Results of this analysis:

Type 1 Analysis of Variance

Source	Error DF	F Value	Pr > F
trt	8.8832	3.93	0.0486
time	3	0.37	0.5862
time*trt	9	4.44	0.0356



Analysis of Hypothetical LTE Data Assuming Linear Time Trends
40
35
30
25
20
15
10
5
0
RESPONSE
1
2
3
4
5
YEAR
TRT1
TRT2
TRT3
TRT4


Hypothetical LTE Trends: True Means
RESPONSE
YEAR
40
35
30
25
20
15
10
5
0
1
2
3
4
5
TRT1
TRT2
TRT3
TRT4

Improved Analysis of LTE data, cont'd

1. Assume that the fixed trends follow a specific pattern

Advantages:

- Interpretation is straightforward
- Can be quite accurate if chosen equation is right (or close)

Disadvantages:

- When chosen equation is wrong:
  - Get wrong comparisons of means
  - Lose power for tests (error terms inflated)
- Need to model serial correlation

Improved Analysis of LTE data, cont'd

2. Assume that "Random" effects can be explained
(Cochran 1939, [Fisher], Patterson 1953, and others)

- Add covariates for each time to explain "random" variation
  - Rainfall (overall or at certain periods within season)
  - Solar radiation/cloud cover
  - Pest incidence
  - Something else
- Use these as additional fixed effects in serial correlation models
- Add interactions with TRT
- *Assume* that *all* random variation is explained by covariate(s)

*Improved Analysis of LTE data, cont'd

2. Assume that "Random" effects can be explained

ANOVA for this analysis (starting with standard split plot):

Source	DF	
Block	$(b-1)$	X_1 = First Covariate
TRT	$(t-1)$	X_2 = Second Covariate
Block*TRT	$(b-1)(t-1)$	(more are possible)
X_1	1	
X_2	1	Year, Year*TRT now *fixed*
Year	$\boldsymbol{(r-1)-2}$	← Lose DF to covariates
$X_1 * TRT$	1	
$X_2 * TRT$	1	
Year*TRT	$\boldsymbol{(r-1)(t-1)-2}$	← Lose DF to covariates
Error	$t(b-1)(r-1)$	
Total	$btr-1$	

*Improved Analysis of LTE data, cont'd

2. Assume that "Random" effects can be explained

Proc Mixed code for this analysis:

```
proc mixed data=set1;
  class block trt year;
  model y = trt X1 X2 Year X1*trt X2*trt Year*trt/ddfm=kr;
  random block block*trt;
  Repeated Year / subject=block*trt type=___;
  lsmeans trt / diff at means;
  lsmeans trt / diff at (x1,x2)=(___,___);
run;
```

Improved Analysis of LTE data, cont'd

2. Assume that "Random" effects can be explained

Advantages:

- "X*TRT" effects can help in understanding TRT reactions to environmental factors

Disadvantages:

- Covariates not likely to capture *all* random variability
  - Remainder contaminates fixed effect (F-tests reject too often)
- Still need to model serial correlation
- Must plan ahead to measure covariates
  (or be able to obtain them retrospectively)

Improved Analysis of LTE data, cont'd

3. Assume that there is a specific amount of variability associated with the random effects.

- Use past history with responses to measure year-to-year variance
- Incorporate specific values for Time and Time*TRT random-effect variance into the model

Dangerous: the *population* values of variance are probably not well known.

Recommendation

Use whatever you are most comfortable with for your problem!

In general a model for the mean trend is probably safest and easiest, as long as a reasonable model can be found.

(Can combine this with other assumptions.)

NOTHING works exactly right. Ignoring the problem is even worse.

How can this problem be avoided?

Problem is a lack of replication of the *time sequence*

- First time measurements all subjected to year 1 environments
- Second time measurements all subjected to year 2 environments
- And so forth

Alleged "replication" (blocks) are really just *subsampling* the same time sequence (pseudo-replication).

SOLUTION:

REPLICATE LTE IN TIME!

This isn't new...

From "Instructions for Authors" for *Agronomy Journal* (www.asa-cssa-sssa.org):

> **"Field experiments that are sensitive to environmental interactions and in which the crop environment is not rigidly controlled or monitored, such as studies on crop yield and yield components, usually should be repeated (over time or space, or both) to demonstrate that similar results can, or cannot, be obtained in another environmental regime."**

Here "environmental regime" = sequence of years!

The Facts

If you want to make statements about time sequences other than the particular one you observe, then you must obtain *replicate time sequences*.

Who wants to wait until an LTE is over to start another one???

The Compromise

The Staggered-Start Experiment

Smith (1979), Preece (1986), McRae and Ryan (1996), Davies (1996)

Staggered Start Design for LTE

Rep	TRT	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	etc
		Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7	Time 8
1	2								...
	5								...
	1								...
	4								...
	3								...
			Time 1	Time 2	Time 3	Time 4	Time 5	Time 6	Time 7
2	1								...
	4								...
	3								...
	5								...
	2								...
				Time 1	Time 2	Time 3	Time 4	Time 5	Time 6
3	3								...
	2								...
	5								...
	4								...
	1								...

Analysis of a Staggered-Start LTE

Derived Variables analysis is “nearly legitimate”

- Effects of year sequences are different in different blocks
- Responses for units in different blocks not quite independent, but not heavily correlated.
- Tests **probably** not badly affected
  (Results as if independent units)

Analysis of a Staggered-Start LTE

Modeling the serial correlation can be done with amended starting ANOVA

- Separate effects for Year (random) and Time (Fixed)
- Certain interactions combine to form error terms
- Works out to be like a weird combination of Latin Square and Strip plot

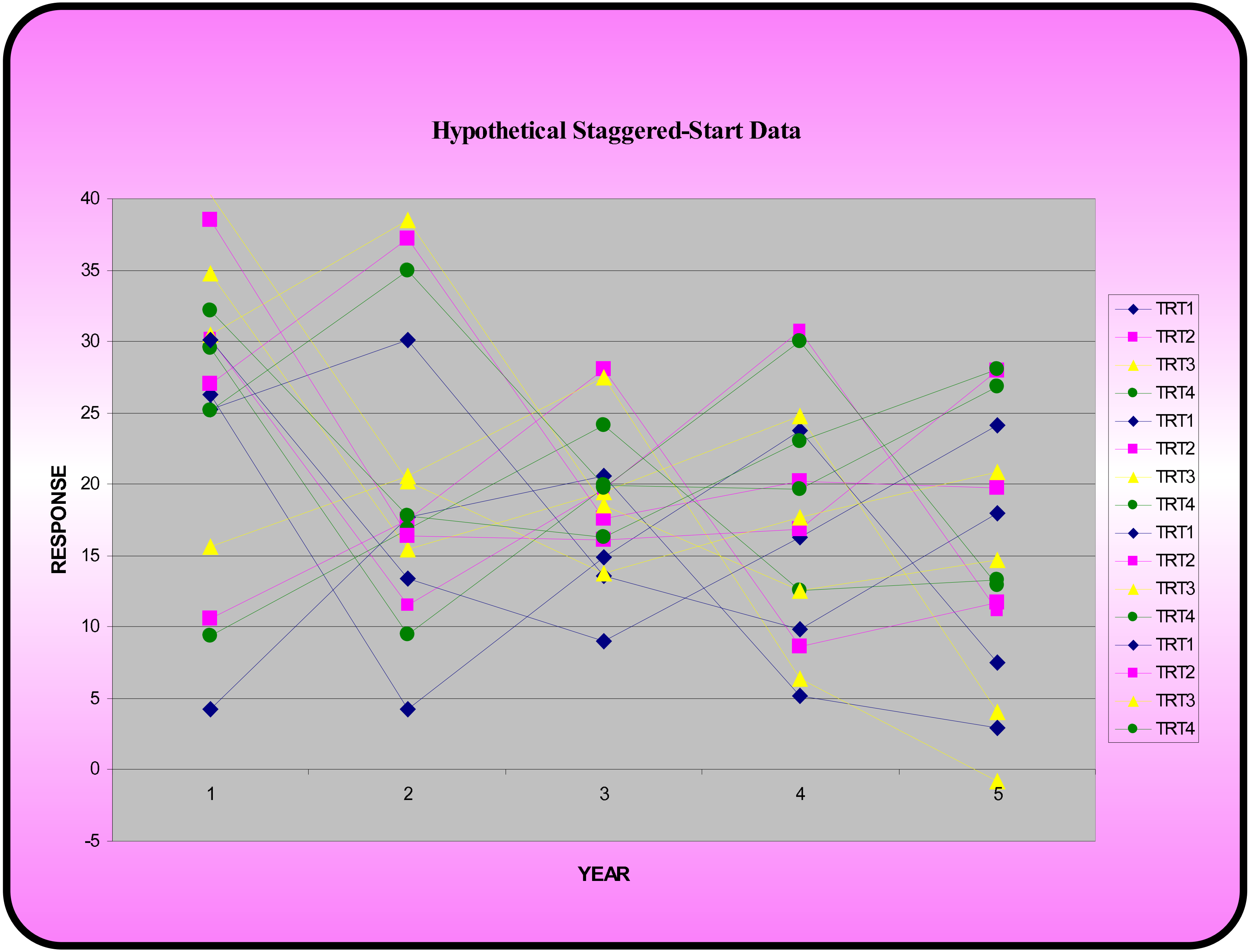
*Analysis of a Staggered-Start LTE

Source	DF	Now
Block	$(b-1)$	# Years > # Times
Year	$(y-1)$	$y = \#$ years
Time	$(r-1)$	$y = t + b - 1$
Blk*Year*Time	$(r-2)(b-2)-1$	
TRT	$(t-1)$	
Block*TRT	$(b-1)(t-1)$	
Year*TRT	$(y-1)(t-1)$	
Time*TRT	$(r-1)(t-1)$	
Error	$(t-1)((r-2)(b-2)-1)$	
Total	$btr-1$	

Analysis of a Staggered-Start LTE

Returning to hypothetical example: Recreated data

- Used same means as before
- Added variability for Year, Year*TRT
- Added block and plot variability
- Ran the analysis


Hypothetical Staggered-Start Data
RESPONSE
YEAR
40
35
30
25
20
15
10
5
0
-5
1
2
3
4
5
TRT1
TRT2
TRT3
TRT4
TRT1
TRT2
TRT3
TRT4
TRT1
TRT2
TRT3
TRT4
TRT1
TRT2
TRT3
TRT4

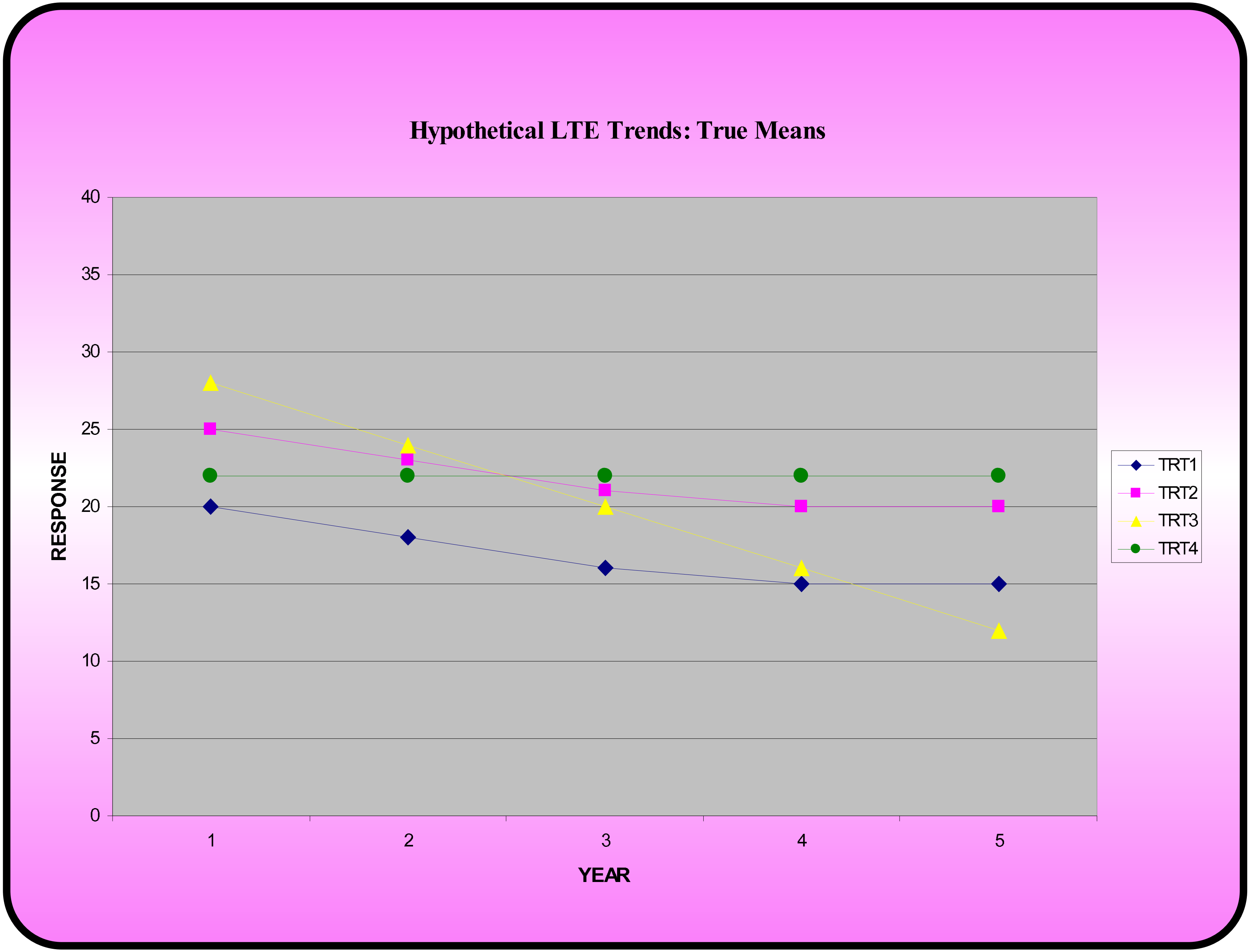
*Analysis of a Staggered-Start LTE

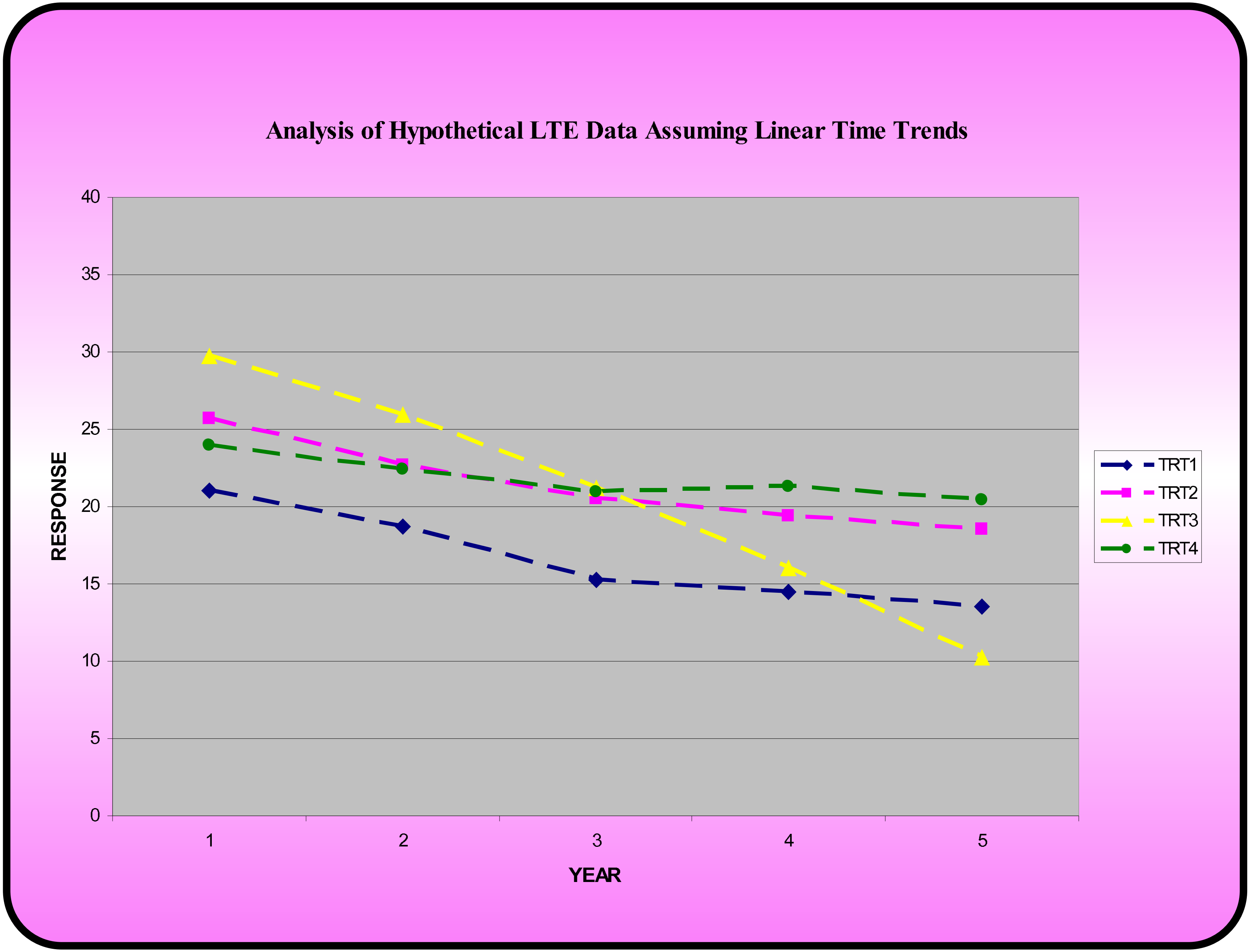
```
proc mixed data=set1;
  class block trt year time;
  model y = trt time time*trt / ddfm=kr;
  random block year block*year*trt block*trt year*trt;
  * Repeated time / type=... <--- not needed here because
  lsmeans TRT*TIME / diff;        no serial corr
run;
```

Analysis of a Staggered-Start LTE

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
trt	3	20.7	17.23	<.0001
time	4	26.7	2.94	0.0387
trt*time	12	36.1	18.01	<.0001


Hypothetical LTE Trends: True Means
RESPONSE
YEAR
0
5
10
15
20
25
30
35
40
1
2
3
4
5
TRT1
TRT2
TRT3
TRT4


Analysis of Hypothetical LTE Data Assuming Linear Time Trends
RESPONSE
YEAR
0
5
10
15
20
25
30
35
40
1
2
3
4
5
TRT1
TRT2
TRT3
TRT4

Conclusions

1. **LTEs are generally designed wrong**
2. **This flaw invalidates ALL standard analyses**
3. **Analyses can be partially salvaged by added assumptions**
4. **Staggered-Start design circumvents problem!**

Slides from talk available at

`www-personal.ksu.edu/~loughin/STATPAGE.html`

*References

1. Bailey, T. B., Swan, J. B., Higgs, R. L., and Paulson, W. H. (1996), "Long-Term Tillage Effects on Continuous Corn Yields," *Proceedings of the 8th Annual Conference on Applied Statistics in Agriculture*, Manhattan, KS: Kansas State University, 18–32.

2. Cochran, W. G. (1939), "Long-Term Agricultural Experiments" (with discussion), *Journal of the Royal Statistical Society*, 6[Suppl.], 104–148.

3. Davies, A. (1996), "Long-Term Forage and Pasture Investigations in the UK," *Canadian Journal of Plant Science*, 76, 573–579.

4. Guerin, L. and Stroup, W. W. (2000), "A Simulation Study to Evaluate PROC MIXED Analysis of Repeated Measures Data," *Proceedings of the 12th Annual Conference on Applied Statistics in Agriculture*, Manhattan, KS: Kansas State University, 170–203.

*References, cont'd

5. McRae, K. B. and Ryan, D. A. J. (1996), "Design and Planning of Long-Term Experiments," *Canadian Journal of Plant Science*, 76, 595–601.

6. Patterson, H. D. (1953), "The Analysis of the Results of a Rotation Experiment on the Use of Straw and Fertilisers," *Journal of Agricultural Science, Cambridge*, 43, 77–88.

7. Poehlman, M. J. (2003), "Design and Analysis of Long-Term Field Trials with Annual Measurements," unpublished M.S. report, Department of Statistics, Kansas State University.

8. Preece, D. A. (1986), "Some General Principles of Crop Rotation Experiments," *Experimental Agriculture*, 22, 187–198.

*References, cont'd

9. Smith, A. (1979), "Changes in Botanical Composition and Yield in a Long-Term Experiment," pages 69–75 in *Changes in Sward Composition and Productivity*, eds. A. H. Charles and R. J. Hagger, Reading, UK: British Grassland Society.