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Trial Designs and Statistics to Obtain Reliable Results

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My background

- **I am an entomologist**
  - PhD in Entomology, minor in experimental statistics
  - I am not a statistician but I use experimental design and statistics to do my job

- **We all face comparable challenges**

A possible scenario

- **Efficacy evaluation**
  - 3 treatments + non-treated check
  - RBD with 4 replications
  - Apply treatments once
  - Collect data from 10 plants/plot 3, 7, and 14 DAT
  - Collect whole plot yield data

- **A potential approach**
  - Get protocol, enter protocol in ARM or Field Pro
  - Conduct trial
  - Enter data in software, run standard ANOVA and separate means with LSD
  - Look at mean separations on each date using letters

Are we using trial designs & statistics that will provide reliable results?

- Why do we have an RBD?
- Why do we have 4 reps?
- Why do we take 10 plants/plot?
- How do we consider the impact of date?
- How do we compare treatments?

- What does this mean?
- Should it be done differently?

Experimental design and data analysis are dependent

The goal of a trial is to compare experimental treatments

- **We measure variables of interest for this comparison** (e.g., yield for each treatment)
- **Variables can describe populations** (e.g., all fields treated by treatments)

- **These variables have a mean (location) and variance (variability)**

The goal of a trial is to compare experimental treatments

- **We cannot measure variables for all individuals in the populations** (e.g., yield in all fields treated by experimental treatments)
- **We work on subsets of populations (samples)** (e.g., trial plots and samples in trial plots)

- **Variables in samples also have means and variances, which are estimates of population means and variances: We can make inferences about populations**

The goal of a trial is to compare experimental treatments

- The sample means are also variables that have distributions with same means as populations and a bell shape

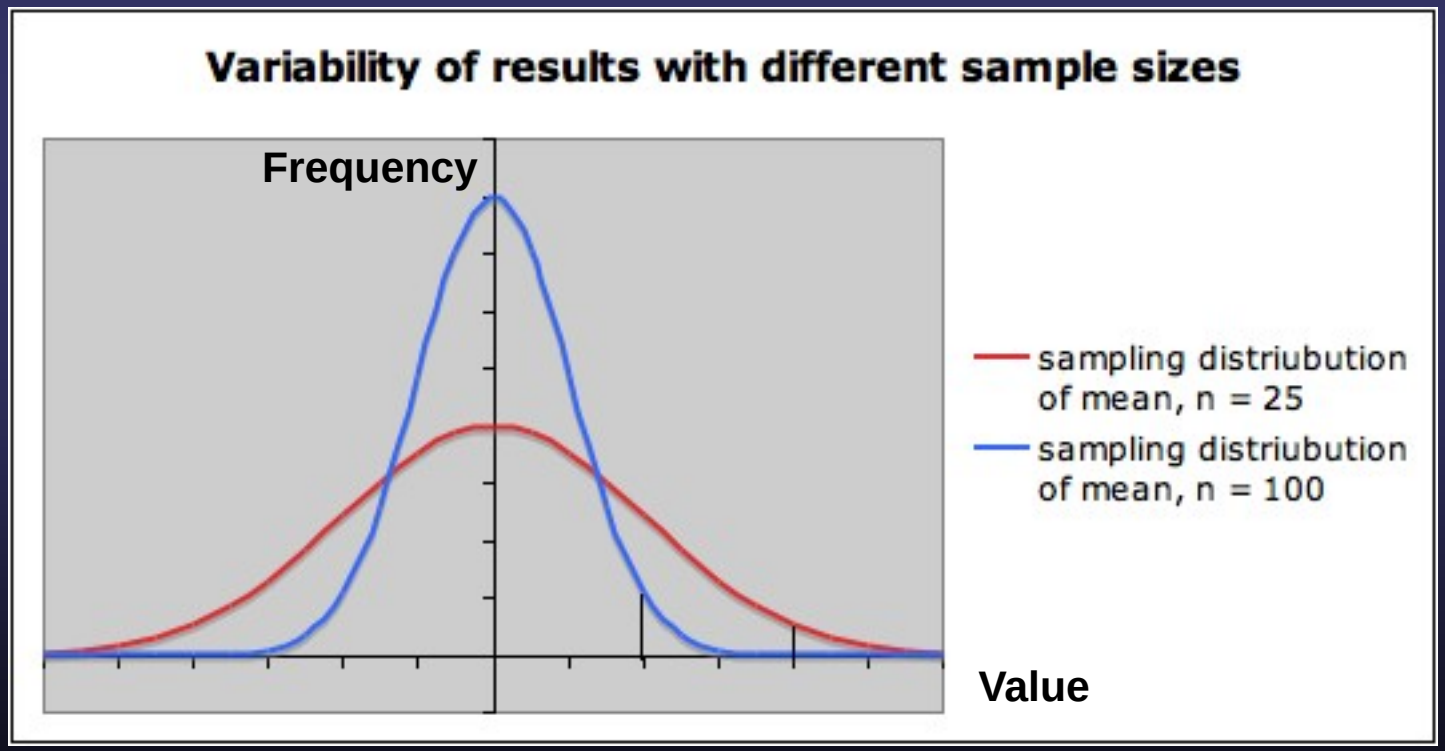
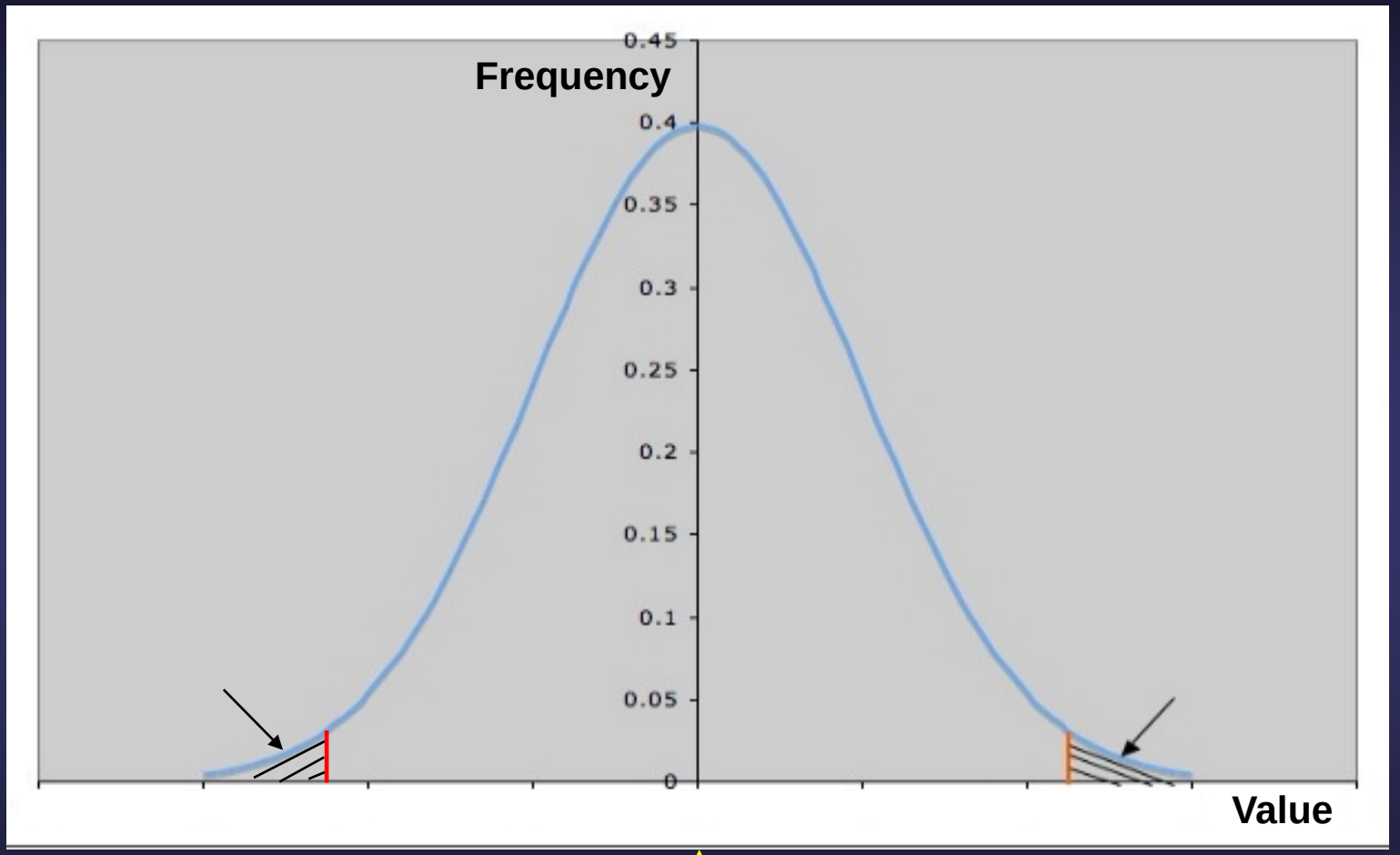


Chart: https://web.ma.utexas.edu

Observed sample mean in a trial



Observed sample mean

Population mean

Sample means

Chart: https://web.ma.utexas.edu

Inference using hypothesis testing

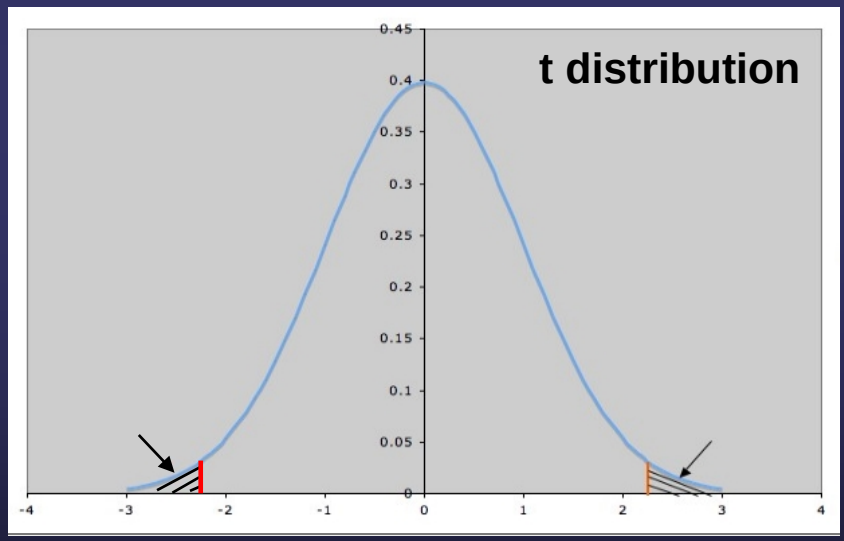
- **Is the population mean different from a hypothesized mean value (e.g, average yield of standard)?**

Null hypothesis H_0: **Mean yield of experimental treatment = Historical mean yield of standard treatment**

t value: a "t value" can be computed using the sample mean and variance, and the hypothesized mean

$$\text{t value} = \frac{\text{sample mean - hyp. mean}}{\sqrt{(\text{variance/n})}}$$

One sample t test: under some assumptions, t value follows a t distribution with n-1 degrees of freedom



t value under H_0

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Inference using hypothesis testing

- **Is the population mean different from a hypothesized mean value (e.g, yield of standard)?**

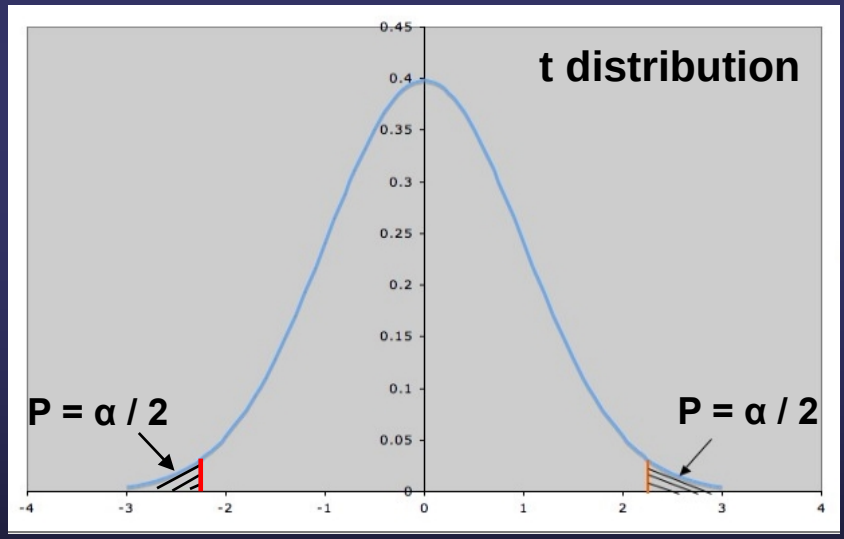
Null hypothesis H_0: **Mean yield of experimental treatment = Historical yield of standard treatment**

Type 1 error: probability of rejecting the null hypothesis while it's true

α: significance level, often 0.05. Probability of type 1 error is ≤ 0.05

P-value: probability of observing the sample mean if H_0 is true

If P-value ≤ α, we reject H_0
Otherwise, we cannot reject.



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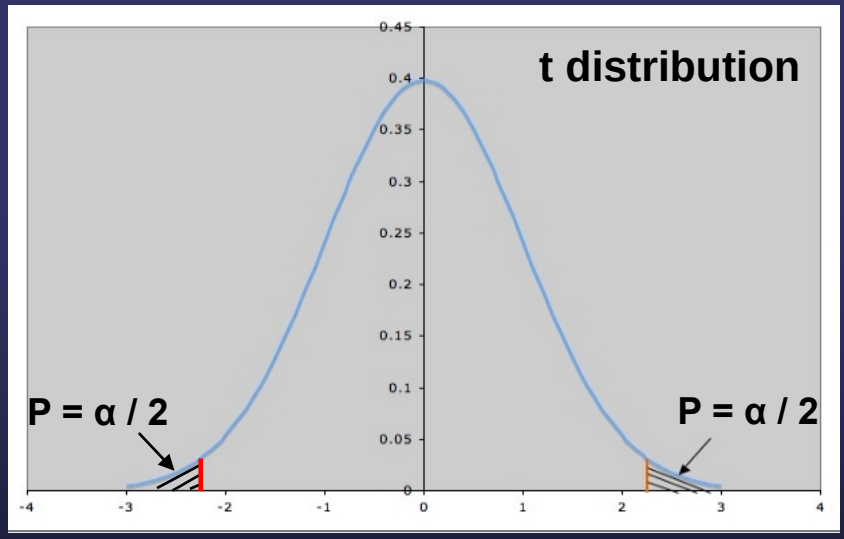

Observed t value

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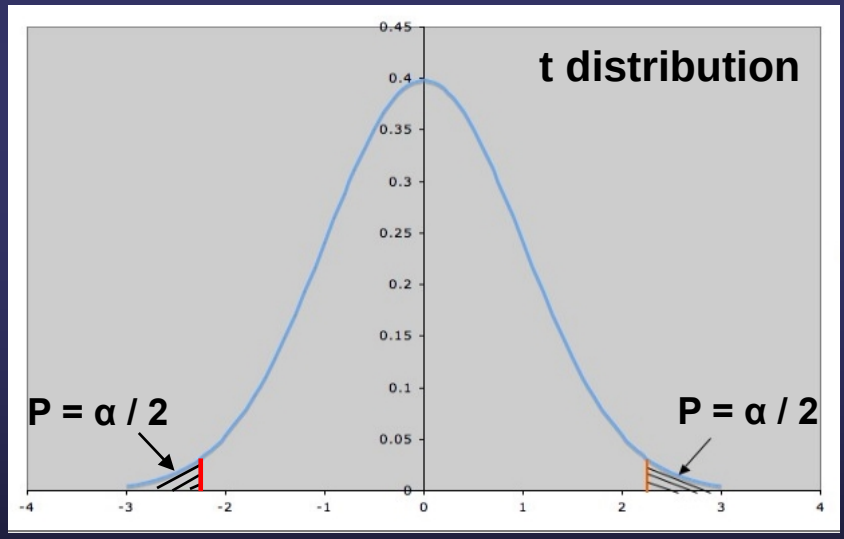

Observed t value

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Inference using hypothesis testing

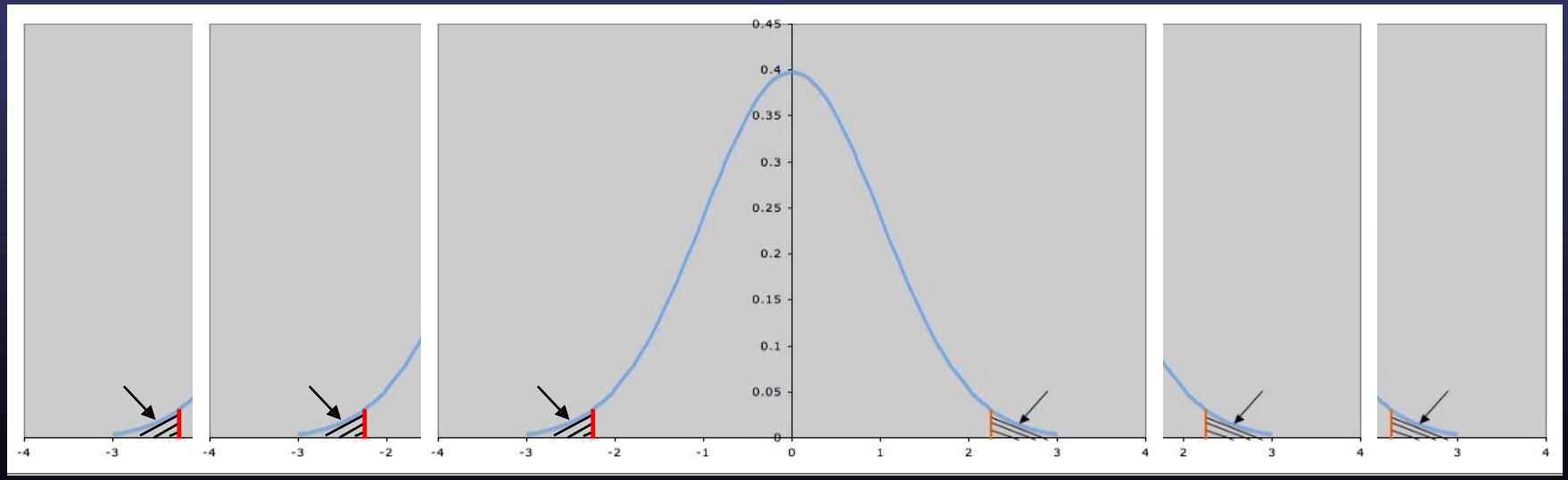
- **Is the population mean different from another population's mean (e.g, yield of standard)?**

Null hypothesis: **Mean yield of experimental treatment = Mean yield of standard**

We test if the difference between the means is equal to 0. We pool the variances between the two samples.

Independent sample t test.

If P-value ≤ α, we reject H_0. Otherwise, we cannot reject.

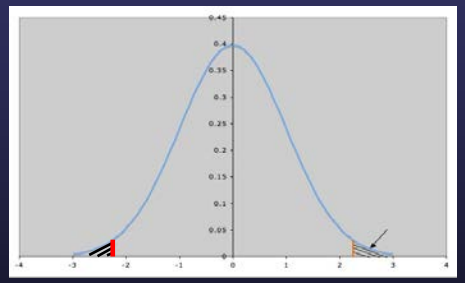
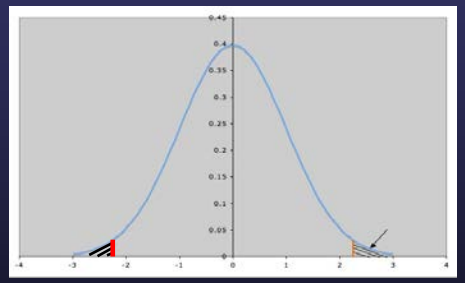
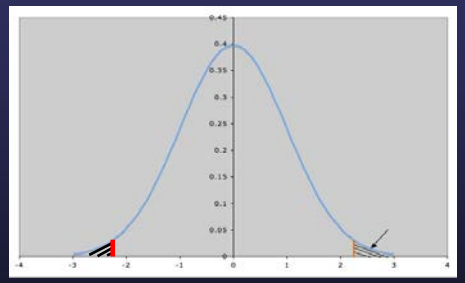
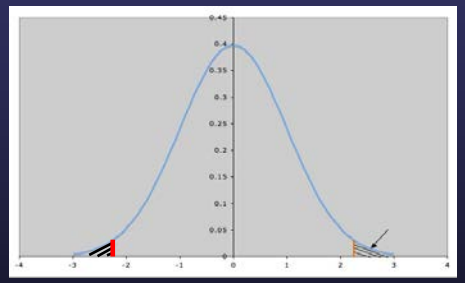


Inference using hypothesis testing

- **Are means from several populations different from each other?**

Null hypothesis: **Mean yields of all treatments evaluated are equal**

We cannot do several pairwise comparisons using independent sample t tests because it substantially increases the overall α

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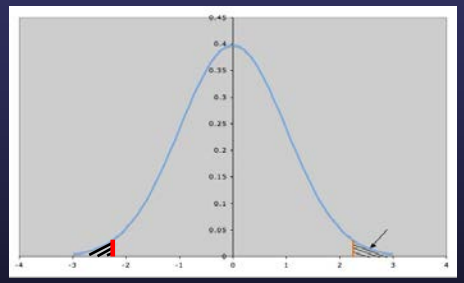
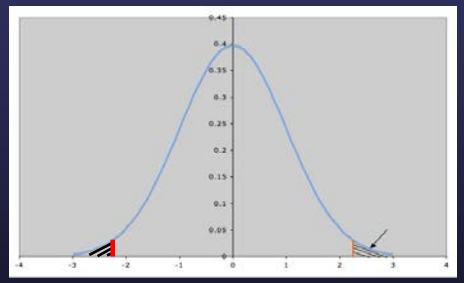
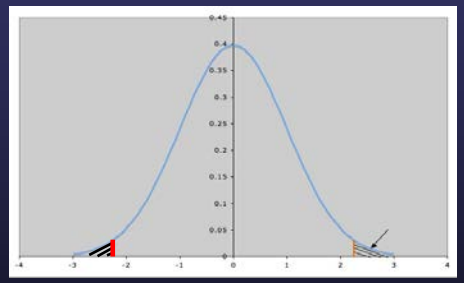
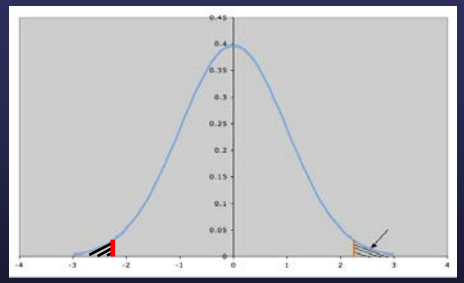
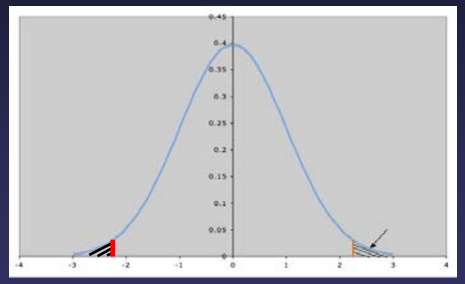
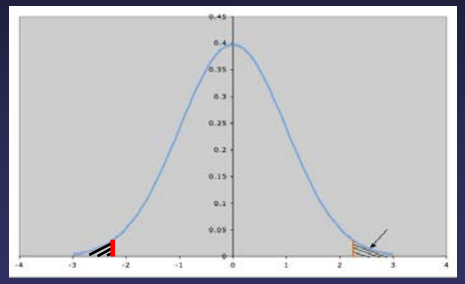
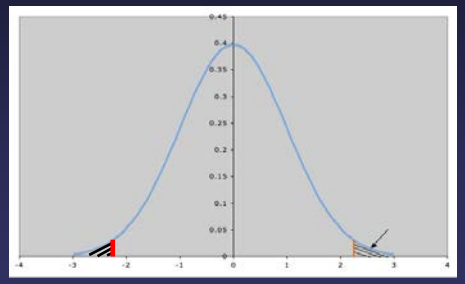
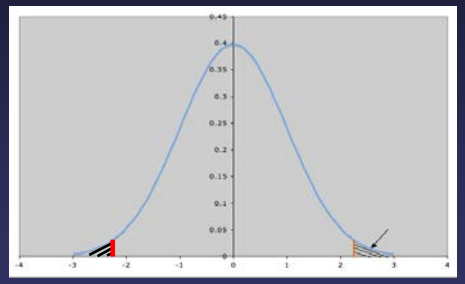
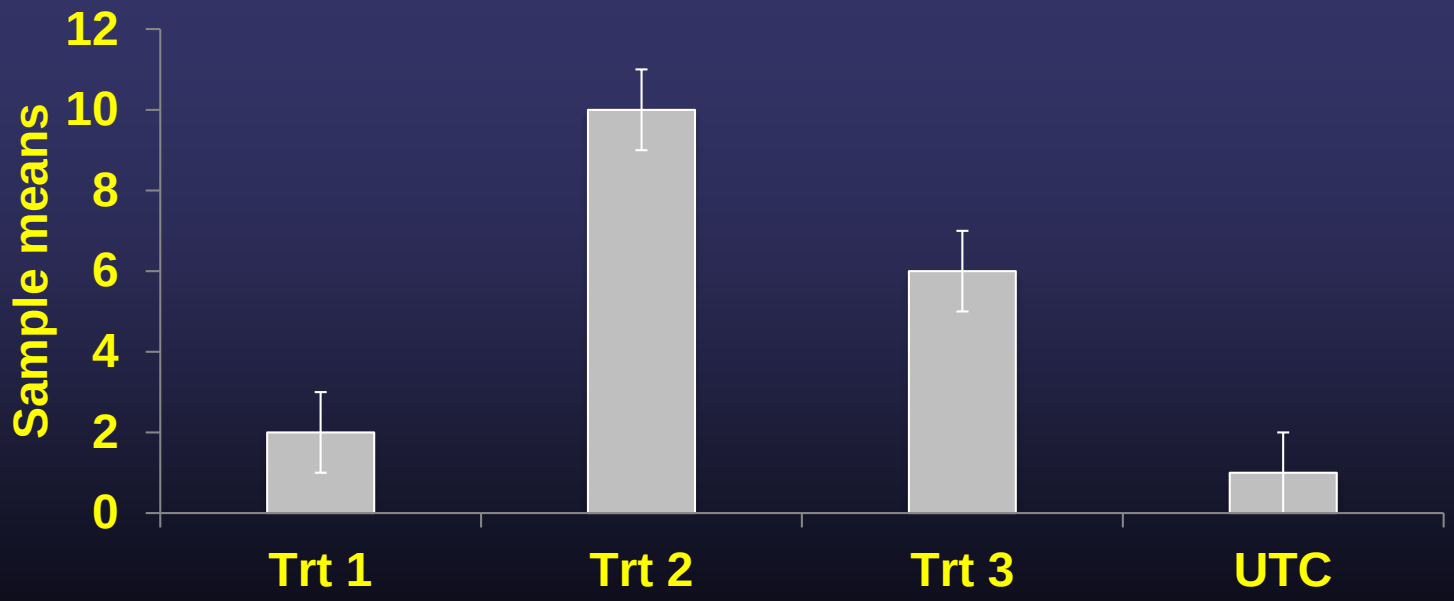
ANOVA. We compare variances.

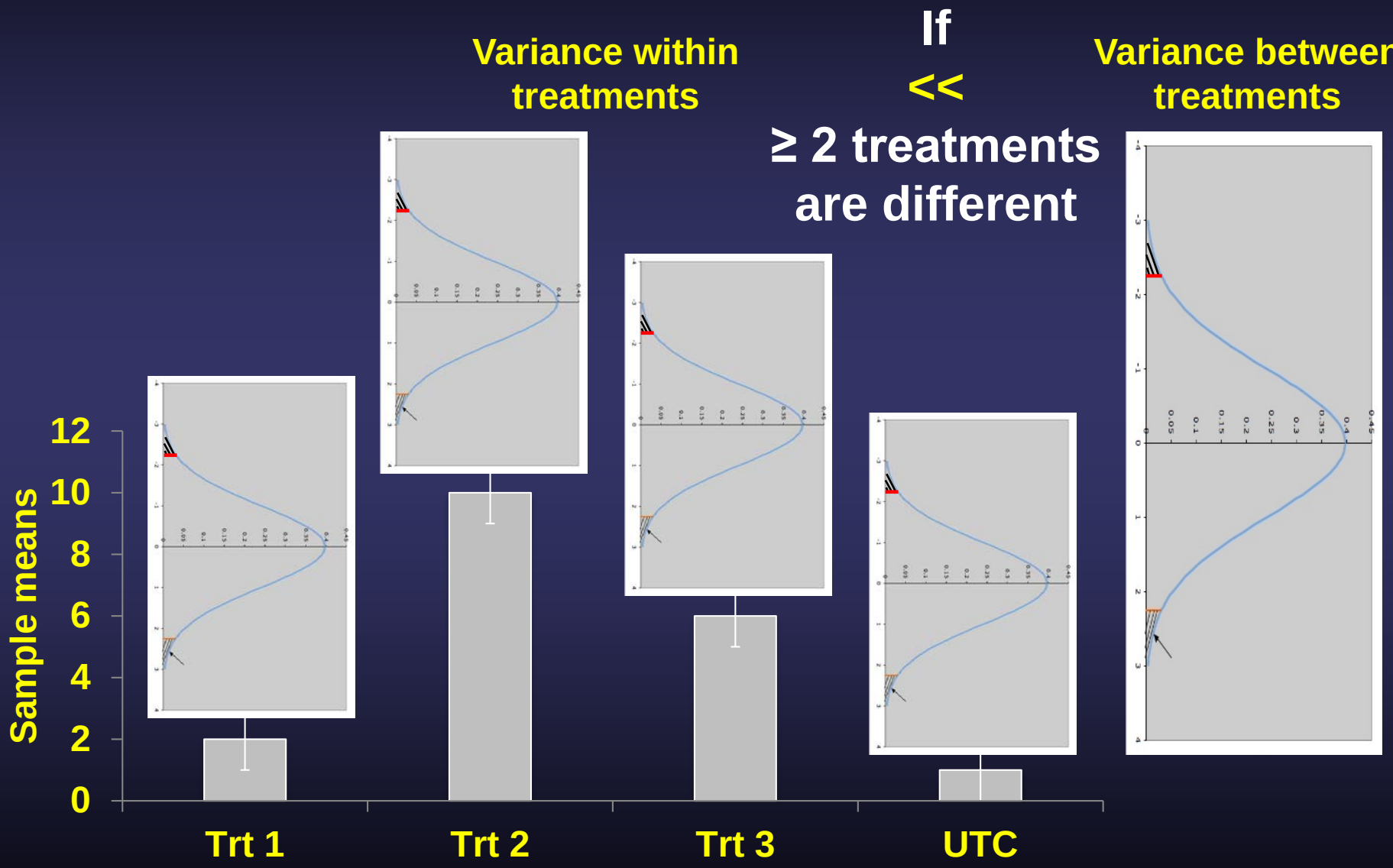





ANOVA

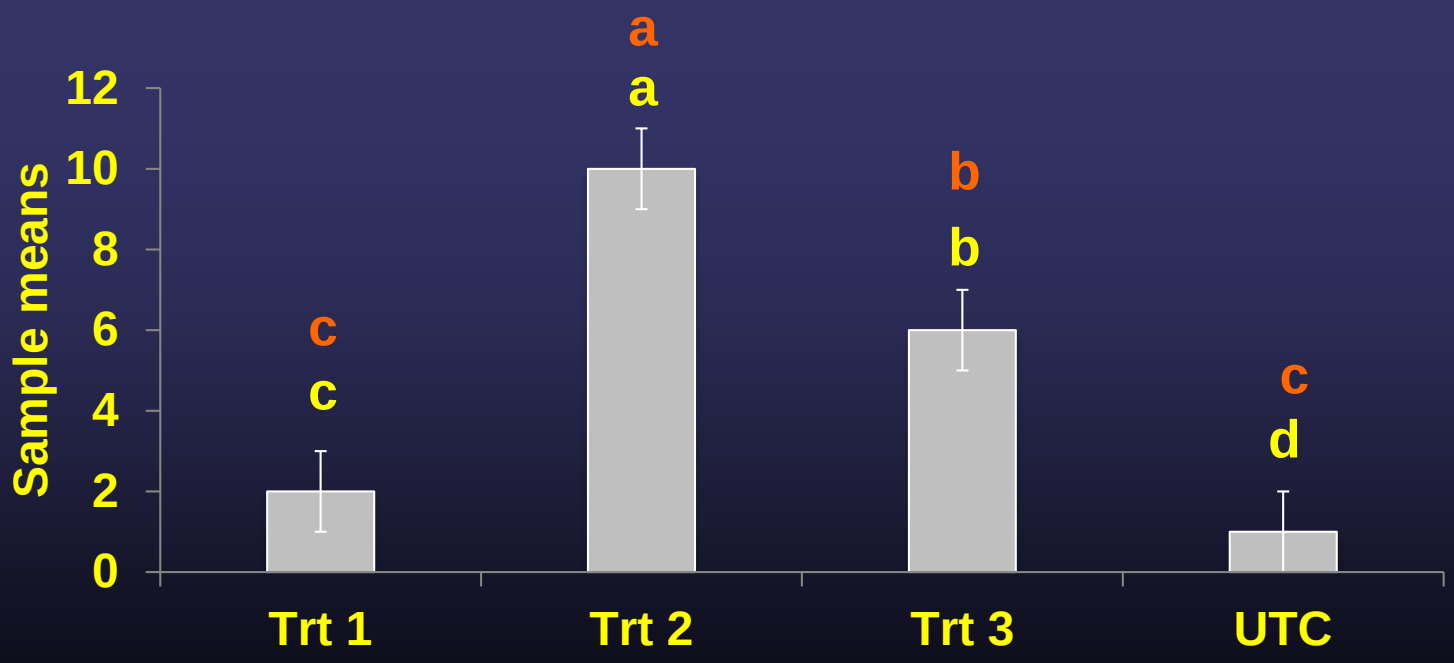


ANOVA


Variance within treatments
If
<<
≥ 2 treatments are different
Variance between treatments
Sample means
12
10
8
6
4
2
0
Trt 1
Trt 2
Trt 3
UTC

ANOVA

- **Variance between treatments / variance within treatments: This is the F value used to determine the P-value**
- **If P-value ≤ α, H_0 is rejected**
- **If H_0 rejected, means are separated using pairwise tests (means with the same letters are not different, P > 0.05)**



Tukey's HSD

LSD

Significant or not significant? That is the question

- **A note on “significance”**
  - **Probability of type 1 error is acceptably low**
  - **α is up to you and your peers**
- **Even though differences are significant, they may not have any biological or agronomic relevance**
- **If differences are not significant, it just means that we cannot detect differences regardless of numerical trends.** **In reality, differences may or may not exist.**
- **Expertise is important**

To compare experimental treatments…

- **We need to estimate means but also variability**
  - **Justification for replications**
- **We need to control variability as much as possible so that variability observed is primarily associated with known factors, including our experimental treatments**
  - **Justification for experimental design and professional experience**

Experimental design

- **Design units**

  - Experimental units: experimental material to which treatments are assigned using randomization (e.g., plots)

  - Sampling units (subsets of the experimental unit) (e.g., rows, plants, fruits within plots)

  - Observation units (unit on which variables are measured)

- **The number of experimental units per treatment is the number of replications**

Number of replications (sample size)

- **How many replications do we need?**
  - Are 4 replications OK? 4 is better than 3. 5 is better than 4
  - There are conventions, practical considerations, and previous experiences
- **Formula based on LSD**

$$LSD = t\sqrt{(2*variance/n)}$$

- If a difference of predetermined value should be detected. **Predetermined difference = LSD**
- If comparable experiments have been conducted in the past. **Variance = variance from past experiments**

$$n = 2*t^2*variance/LSD^2$$

$$t \approx 2.3$$

Experimental design: Blocks

- **CRDs and RBDs**
  - **What are blocks?**
    Groups of experimental units (not independent)
  - **Group can be in space or time**
- **Why using blocks in RBDs?**
  - **Control of variability**
- **Other designs use blocks**
  - **Latin square design (same no. treatments and blocks, 2 block structures)**

Experimental design: Split plot designs

- **It's all about experimental units**
  - Split plots can be CRDs, RBDs, or Latin squares
  - Used when 2 factors are studied (e.g., irrigation and variety, 3 levels each)

- 1 factor randomized to experimental units (main plots)
- 1 factor randomized to subsets of experimental units (sub plots)
- Alternative: conventional factorial design in which combinations of 2 factors are randomized to experimental units (plots)

- Why using split plot designs?
  Practical considerations

Experimental design: Repeated measures

- **Exactly same variable measured on exactly the same piece of experimental material over time or space**

- **Reduced number of experimental material** (no need for different material over time or space)
- **Reduction of variability associated with experimental material** (same used throughout study)

- **Observations are not independent** (correlated within experimental material)

The ANOVA assumptions

- ANOVA assumes normal distribution, homogeneity of variance, independence of samples
- Assumptions are often violated
- What do we do?
  - Keep it simple
  - Have a good experimental design, ANOVA model

The skills and experiences of research staff are extremely important

Trial Designs, Statistics to Obtain Reliable Results

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