Twigs and the T-Test

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Introduction

Do you want to introduce statistical analysis of data into a botany lab? This can be done easily enough in a lab on twig structure and twig growth.

First, the students learn basic twig anatomy by examining twigs of several tree species while referring to a figure of a labeled twig, such as the figure in the Appendix. After learning the various twig structures and their functions, the students practice finding the bud scale scars on twigs. The number of bud *scales* per bud in a species will determine how obvious the bud scale *scars* will be on a twig.

Does a twig grow the same length in each year? After reviewing the process of twig growth, each student measures the length of twig growth produced during each of the last two completed growing seasons on a randomly selected twig. These measurements generally indicate that a twig does not have exactly the same amount of growth each year.

Next, for the tree overall, is there a "significant difference" in twig growth between the past two completed growing seasons? The instructions and data sheet for the students to measure twigs and pool their data are in the Appendix. Each student can take the two measurements on more than one twig in order to get a large sample size when the class data is pooled. The sheet of pooled data is then photocopied and distributed to all of the students.

Finally, in preparation for the data analysis, students are taught about the basics of two-sample as compared to paired-sample t-tests, as well as two-tailed as compared to one-tailed hypotheses; see the Appendix. It should be mentioned to the students that rigorous analysis would require the performance of an F-test, to test for equality of variances in the two sampled populations, which is an assumption of the t-test; however, the F-test could be omitted here because the t-test is robust especially with large, equal sample sizes and the two-tailed hypothesis being used in this investigation. The students may perform the paired-sample two-tailed t-test by hand or they may use Microsoft® Excel® (or other statistical software) as described in the Appendix.

Instructor's Notes

- A good reference for brushing up on statistics is Biostatistical Analysis, Fourth Edition, by Zar (1999); note that the fifth edition is in preparation.
- Each twig selected by the students should have at least two complete years of growth on it.
- The instructor needs to monitor the students to make sure that the bud scale scars are being correctly identified.
- Tree species that have buds with *numerous* bud scales will produce the most obvious bud scale scars; thus, for example, an oak or a beech works well in this investigation.
- It is more challenging for beginning students to measure maple twigs correctly because the two oppositely-positioned leaf scars form a scar encircling the twig; this encircling scar tends to get misread as bud scale scars. If such a tree is selected for the investigation, then the instructor must be even more diligent at monitoring the students' identification of bud scale scars.
- The instructor also needs to make sure that students are all using the same scale (metric) on their rulers.
- The statistical analysis could be done during a different class session; at that time, the graphing of the data as superimposed histograms could also be done.

Literature Cited

Tschunko, A. 2007. Plant Biology Laboratory Manual. Pearson Prentice Hall, Upper Saddle River, New Jersey, 249 pages.

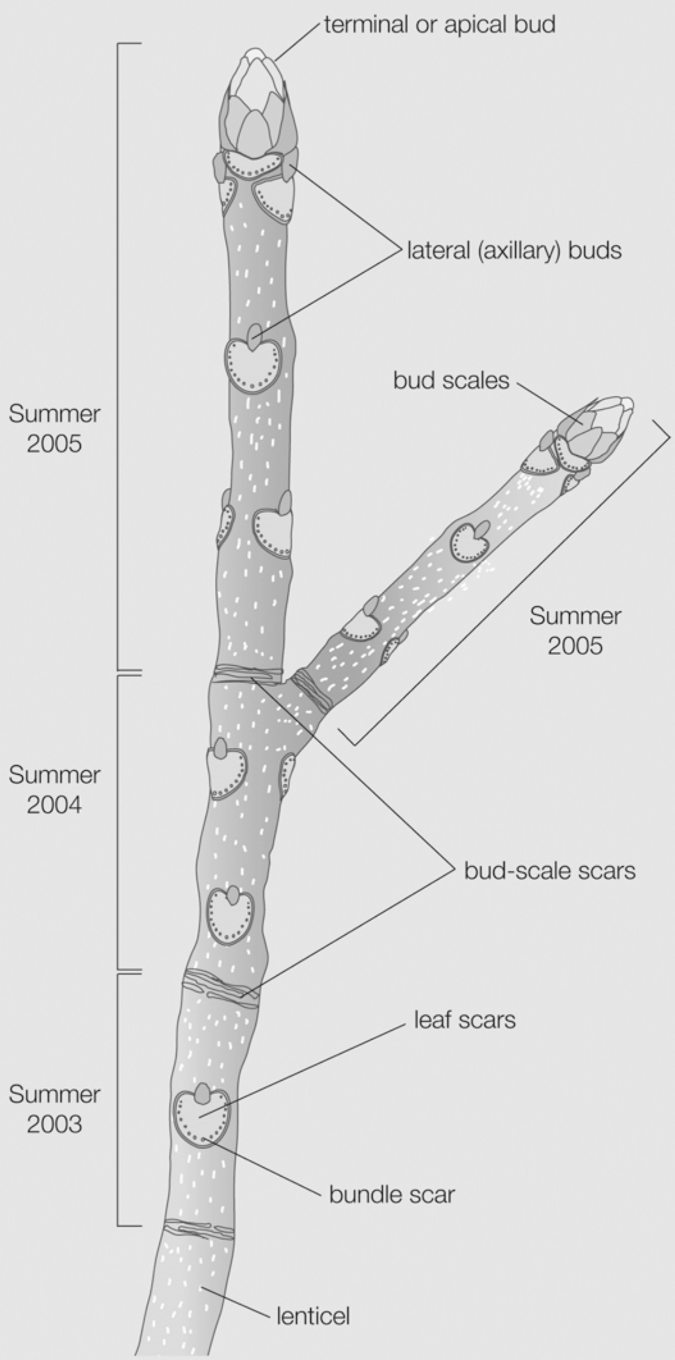
Zar, J. H. 1999. Biostatistical Analysis, Fourth edition. Pearson Prentice Hall, Upper Saddle River, New Jersey, 931 pages.

About the Author

Almuth H. Tschunko received her B.S. from Tufts University, M.A.T. from Cornell University, and M.S. and Ph.D. from University of Michigan. She is a professor in the Biology Department of Marietta College where she teaches botany courses with field trips, as well as genetics and various introductory biology courses. She wrote the book Plant Biology Laboratory Manual, for Pearson Prentice Hall.

Appendix

From: Tschunko, A. 2007. Plant Biology Laboratory Manual. Pearson Prentice Hall, Upper Saddle River, New Jersey, 249 pages. Figure 9.1 of page 88 and pages 91, 240, 244, and 245 are reprinted here by permission of Pearson Education, Inc.



Terminal or apical bud
- Contains miniature unelongated shoot
- The twig grows in length when its terminal bud grows

Lateral or axillary bud
- Grows into a side shoot (lateral shoot)
- Present in every leaf axil, and above every leaf scar

Bud scales
- Tough, shingle-like modified leaves covering a bud
- Protect the bud contents against damage and desiccation

– Oak bud has many bud scales
– Birch bud has several bud scales
– Willow bud has one cap-like bud scale
– Pawpaw bud is a miniature hairy shoot with no bud scales

Bud scale scars
- Thin scars left behind when bud scales fall off in the spring

One year's growth
- The length of twig from a terminal bud back to the first set of bud scale scars
- The length of twig between two sets of bud scale scars

Leaf scar
- Scar left behind when a leaf falls off the twig

– Maple twig has narrow, V-shaped leaf scars
– Buckeye twig has large, shield-shaped leaf scars

Bundle scar
- Within a leaf scar
- The end of a vein (vascular bundle) that had entered the leaf

Lenticel
- Wart-like break in the twig surface
- Allows gas exchange
- Is linear, across the twig in birch and cherry
- Is a round dot in buckeye and maple

Figure 9.1 Anatomy of a twig, collected in autumn of 2005

I.C. TWIG GROWTH

In Part I.B, you probably noticed that each twig grew a little differently and that it can be difficult to determine whether there was a real difference overall in growth during one year as compared to another year. In science, when you want to determine whether there is a significant difference between two groups, you sample each group and then perform a statistical test on the data to determine whether there is a significant difference. The following investigation will give you practice in doing just that.

The purpose of this investigation is to determine whether a particular tree produced the same amount of growth in each of the last two complete growing seasons, or whether there was a significant difference in growth between those two years.

MATERIALS

- rulers (metric)
- trees outside

INVESTIGATIONS

☐ 1. The class will select a tree with easy-to-reach and easy-to-read twigs.

☐ 2. Each student will measure one or two twigs (depending on class size) to determine the length of twig growth in each of the past two full growing seasons. Record your data.

☐ 3. Compile the class data in the following class data sheet.

☐ 4. Each student will group the lengths into *size categories* (categories must be of equal size) and draw a bar graph (histogram) of the data for each year. Put both histograms superimposed on the same set of axes to facilitate visual comparison of the data (see Appendix 2, "Data Presentation"). Your instructor will tell you whether to do this by hand or whether the class will go to the computer lab to use a graphing software program such as Microsoft® Excel.

☐ 5. Determine whether there is *a significant difference* in growth between the two years. To determine this correctly, you need to do a *paired-sample t-test*, because each measurement of one year is associated with (paired with, taken from the same twig as) one and only one of the measurements of the other year. You may have noticed that on a branch, the leader twig tends to grow more than the lateral twigs. See Appendix 3 for instructions on the t-test, performed by hand and by Microsoft® Excel®.

CLASS DATA SHEET: Twig Growth

Twig Number	Length of Twig (cm) grown in year: ________	Length of Twig (cm) grown in year: ________
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
Mean (average) length		

☐ 6. If you find a significant difference in growth between the past two years, then research the environmental conditions during those two growing seasons and propose a factor that might have caused this difference in growth.

☐ 7. Summarize your findings in a report.

II. THE T-TEST (by hand and by Microsoft® Excel®)

(For greater detail, see Zar 1999.)

- Use the *two-sample t-test* (see Parts II.A. and II.B.) when each datum in one sample is not associated in any way with any particular datum in the other sample; this test assumes that the samples are *random*, from 2 *normal* populations having *equal variances*. (See the "Important Notes" below.)
- Use the *paired-sample t-test* (see Parts II.C. and II.D.) when each datum in one sample is in some way associated with (paired with) one and only one particular datum in the other sample; this test does not assume normality and equal variances of the sampled populations. This test *does* assume that the subjects are randomly selected, and that the differences of the pairs in the population are normally distributed.
- For the final step in a t-test, you *must* know if the hypothesis you are testing is "two-tailed" or "one-tailed."

IMPORTANT NOTES:

- The **t-test is "robust"**: It still works if there is considerable departure from the assumptions, especially if:
  - The two sample sizes are nearly equal.
  - The hypothesis being tested is two-tailed.
  - The sample size is large (Zar 1999, p. 127).
- **Variations** on the t-test formula have been developed for use with populations having *un*equal variances. The Microsoft® Excel® "Data Analysis" menu includes a two-sample t-test assuming equal variances as well as a two-sample t-test assuming unequal variances.
- **Nonparametric tests** exist for use with populations that deviate severely from the t-test assumptions.

TWO-TAILED VERSUS ONE-TAILED HYPOTHESES

As you will see later, the t-statistic is calculated the same way for two-tailed and one-tailed hypotheses, but the *t-critical value* (derived from Table A3.1 and compared to the calculated t-statistic) is different for two-tailed and one-tailed hypotheses. Thus, you must know whether you are investigating two-tailed or one-tailed hypotheses.

Investigation with Two-Tailed Hypotheses

The question: Was twig growth in 2002 *different* from (*either* better *or* worse than) twig growth in 2001?

Hypothesis = H_0 = the two population means are equal ($\mu_1 = \mu_2$).

Alternative hypothesis = H_A = the two population means are *different* (*either* $\mu_1 < \mu_2$ or $\mu_1 > \mu_2$).

- *Two-tailed* hypotheses: Investigate whether one population differs in *either direction* from the other population.

Investigation with One-Tailed Hypotheses

The question: A tree received fertilizer in spring 2002; was twig growth in 2002 *better* than in 2001?

Hypothesis = H_0 = the mean growth in 2002 is not better; the mean growth in 2001 is greater than or equal to the mean in 2002 ($\mu_1 \geq \mu_2$).

Alternative hypothesis = H_A = the mean twig growth in 2002 is *better*; the mean of 2001 is less ($\mu_1 < \mu_2$).

- *One-tailed* hypotheses: Investigate whether one population differs in *one direction* from the other population.

OVERVIEW OF THE TWO-TAILED T-TEST

1. The t-test uses the data of both samples to calculate a t-statistic value.
2. The greater the difference between the two samples, the larger the calculated **t-statistic** value will be.
3. The larger the t-statistic value calculated from the two *samples*, the less likely it will be that the two sampled *populations* have identical means.

II.C. PAIRED-SAMPLE, TWO-TAILED T-TEST PERFORMED BY HAND

Use the *paired-sample t-test* when each datum in one sample is in some way associated with (paired with) one and only one particular datum in the other sample.

Example: In a random sample of 20 twigs, two measurements were taken on each twig—the length of 2001 growth and the length of 2002 growth. The sample data are used to test these two-tailed hypotheses about the populations:

Hypothesis = H_0 = in the twig population, there is no difference between twig growth in 2001 and 2002 ($\mu_1 - \mu_2 = 0$).

Alternative hypothesis = H_A = in the twig population, there is a difference between twig growth in 2001 and 2002 ($\mu_1 - \mu_2 \neq 0$).

1. Calculate:
   - The difference between the two measurements of each pair:
     $d = X_1 - X_2$
   - Number of pairs of data = the number of differences = n
   - Mean of the differences = average of the calculated d values = $\bar{d}$
   - Degrees of freedom (df) = ν (Greek letter nu) = $n - 1$
   - Sum of squares = SS = for each calculated difference (d_i), subtract it from the mean of the differences ($\bar{d}$); then square this amount, and then add all these squared amounts together

     $$SS = \sum (d_i - \bar{d})^2$$

   - t-statistic =

     $$\frac{\bar{d}}{\sqrt{\frac{SS}{n(n-1)}}}$$

Table 1. Twig length (cm)

2001	2002	$d = X_1 - X_2$
5	4	1
5	4	1
5	3.8	1.2
5	4.6	0.4
4	4.2	-0.2
4.5	2.5	2
4.6	3.4	1.2
5.7	3.5	2.2
5.1	2.9	2.2
5.3	2.7	2.6
3.9	4.2	-0.3
5.9	4.1	1.8
4.9	4.8	0.1
4.3	5	-0.7
4	3.9	0.1
5.5	4.1	1.4
5	4	1
5.5	4	1.5
4.5	5.2	-0.7
5	3	2

2. If the calculated t-statistic is a negative number, use its absolute value (i.e., ignore its negative sign).
3. Now compare the absolute value of this t-statistic to the tabled t-critical value for your degrees of freedom and $\alpha = 0.05$, two-tailed [$\alpha(2) = 0.05$ in Table A3.1].
5. If the calculated t-statistic is *greater than* the tabled t-critical value, then *reject* the hypothesis that these two samples came from populations with *identical* means. "There *is* a significant difference at the 0.05 level."
6. If the calculated t-statistic is *less than* the tabled t-critical value, then *accept* the hypothesis. "There is *no* significant difference at the 0.05 level."

> *NOTE*: For *one-tailed* hypothesis where the hypothesis H_0 is that the mean population difference is less than or equal to the number **a**, and the H_A is that the mean difference is greater than **a**: the paired-sample t-statistic is calculated with the numerator being ($\bar{d}$ - **a**), and the t-critical value used is for $\alpha(1) = 0.05$ in Table A3.1. As usual, if your calculated t-statistic is greater than the tabled t-critical value, reject H_0.

II.D. PAIRED-SAMPLE, TWO-TAILED T-TEST PERFORMED BY EXCEL® SOFTWARE

Example: In a random sample of 20 twigs, two measurements were taken on each twig—the length of 2001 growth and the length of 2002 growth. The sample data are used to test these two-tailed hypotheses about the populations:

Hypothesis = H_0 = in the twig population, there is no difference between twig growth in 2001 and 2002 ($\mu_1 - \mu_2 = 0$).

Alternative hypothesis = H_A = in the twig population, there is a difference between twig growth in 2001 and 2002 ($\mu_1 - \mu_2 \neq 0$).

In Microsoft® Excel®, type in your columns of data. Then in the Excel® "Tools" menu, click on "Data Analysis" to see a list of statistical tests. (If Data Analysis does not appear on the Excel® Tools menu on your computer, click on "Add-Ins" on the Tools menu instead and install "Analysis ToolPak"; after installation, you should find Data Analysis listed on the Tools menu.) From the list of tests, select "t-Test: Paired Two Sample for Means." Fill in the required information, using the Help button if needed.

Example Printout from Excel®

This t-test was performed on a sample of 20 twigs on which both the 2001 and the 2002 growth were measured. (Note that this example uses the same fictitious data set as was used for Part II.B.; this allows you to compare the outputs of these two kinds of t-tests. This data set is shown in Part II.C. and was also used in Appendix 2 for the histogram.)

t-Test: Paired Two Sample for Means

	2001	*2002*
Mean	4.885	3.895
Variance	0.316078947	0.53313158
Observations	20	20
Pearson Correlation	-0.21302558	
Hypothesized Mean Diff.	0	
df	19	
t Stat	4.374998348	
P(T<=t) one-tail	0.000162863	
t Critical one-tail	1.729132792	
P(T<=t) two-tail	0.000325727	
t Critical two-tail	2.09302405	

Degrees of freedom

Take the "absolute value" of the t-statistic. (This means ignore a minus sign if present.)

There is a 0.0003 chance of getting a t-stat absolute value of 4.375 or larger when hypothesis H_0 is true.

Conclusion:

- Absolute value of t-statistic (4.375) *is greater than* the t-critical two-tailed value (2.093).
- Thus, *reject* the hypothesis that these two samples come from populations with identical means.
- The chance that the two *sample* means are as different as these are, when in fact the two *population* means are identical is less than 5% (the chance here is 0.0003, or 0.03%).
- Conclude: "There *is* a significant difference at the 0.05 level."

In other cases:

- If the absolute value of the t-statistic had been *less than* the t-critical two-tailed value, then the hypothesis that these two samples come from populations with identical means would be *accepted*.
- In such a case, there would be a *better than 5% chance* of getting such sample means when in fact the two population means are identical.
- Conclude: "There is *no* significant difference at the 0.05 level."