Habanero Pepper Germination Referee 2012-2013
Southwest Region IV

INTRODUCTION: At the 2012 AOSA/SCST Referee buzz session, the Southwest Region IV agreed to conduct a germination referee on habanero pepper (*Capsicum chinense*). It was noted that habanero peppers can be difficult to evaluate and may be different in their germination criteria and characteristics from other peppers.

OBJECTIVES: The objectives of this referee were to investigate the current AOSA Rules for habanero peppers. Specifically, we wanted (1) to compare the current 14 day final count to an extended 21 day count, (2) to compare the current 6 day count with a 10 day count, and (3) to compare the use of H_2O with GA_3 which is listed under the 'Fresh and dormant seed' column in Table 6A of the AOSA Rules, Volume 1. The intent was to gather information that might lead to an AOSA rules proposal if warranted.

MATERIALS AND METHODS: Two samples with 1000 seeds each (Samples A and B), along with a written survey, were sent out to 13 laboratories, with following instructions:

1. Please fill out the survey and send back with your results.
2. Please use the media that you typically use in your lab for habanero peppers.
3. Plant 400 seeds of each sample using H_2O as the moistening agent.
4. If possible, plant another 400 seeds in the same media but using GA_3 (500 ppm) as the moistening agent.
5. Use 20-30°C germination temperature.
6. Follow the AOSA Rules seedling evaluation guidelines for peppers.
7. Record 6-day, 10-day, 14-day, and 21-day counts. Use the emailed Excel spreadsheets if possible.
8. Return results by **August 31, 2012** via email or post.

It was difficult to obtain habanero pepper seeds in the quantities needed for this referee; in the end, we were only able to come up with two different samples of differing quality. However, we did get good participation, with 12 laboratories from 4 different states. Three of the labs used H_2O only and did not use GA_3.

SURVEY RESULTS: The following is a summary of the survey and the comments received from the 12 labs:

2012-2013 Habanero Pepper Germination Referee Survey

1. Name of Lab: *Summary – Responses from 12 labs*

2. Do you test habanero peppers for germination in your lab? *-- Yes = 10 labs, No = 2 labs*

3. If yes, estimate how many samples per year: *-- varied from less than 1 to 60+; average 10*

4. List the germination media typically used for habanero peppers in your lab: *-- 8 labs = T; 2 labs = TB*

5. Do you use GA3 for habanero peppers? *-- Yes = 1 lab; No = 9 labs*

6. Do you test standard (non-habanero) peppers in your lab? *-- Yes = 12 labs, No = 0 labs*

7. If yes, do you notice any difference between habanero pepper germination

   and standard pepper germination?   *-- Yes = 8 labs, No = 2 labs*

   Please explain or describe:
   > *-- Habaneros seem a little slower; more difficult to evaluate; slow initial germination; germinate slower, especially the roots; perform much better with GA3; can be more dormant; usually don't germinate as well as regular peppers; like warmer temperature, seem to take longer than 14 days to optimum germination; both kinds have to extend the tests past the final count day*

8. Other comments:
   > *-Planted at 45° angle (two labs); usually extend habaneros to 21 days and report both 14 day and 21 day counts; have found that 50/50 mix of KNO3 and GA3 works very well to break dormancy; only use GA3 if dormancy is suspected at end of test; some habanero samples have more flat seeds (depleted endosperm due to density grading?)*

   Additional Comments from answer sheets:
   > *-- GA3 was slightly faster than H2O test; Lots of borderline sprouts; More abnormals with GA3, but GA3 test was faster; More rapid development with GA3; We don't count peppers on 6 days, too small, waste of time; Dead could be dormant; more difficult to see roots in flat media; 45° slanted test; Abn: fork, stunted root, decayed cots, ungerm . seeds, some very flat seeds and some rotten seeds; did not use GA3 in this lab; stunted root; damaged hypocotyl; pathogen at 10 day count; reps out of tolerance (GA3 test)*

GERMINATION TEST RESULTS:

Sample A:

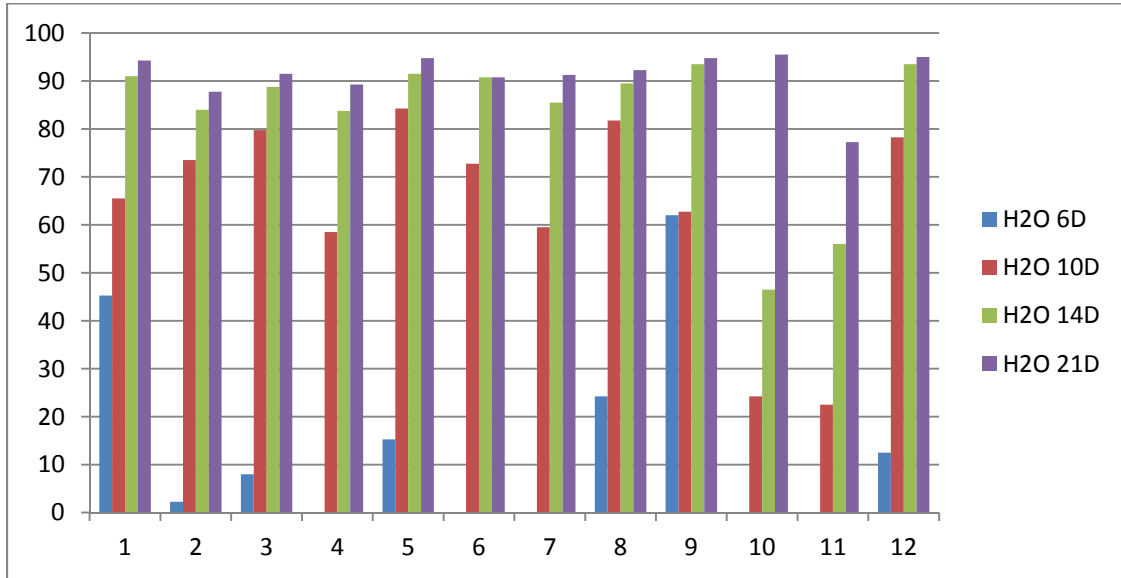| SAMPLE A: Average results for 12 laboratories		
Count	H_2O	GA_3
6 day	14.13%	10.97%
10 day	63.60%	71.39%
14 day	82.85%	86.39%
21 day	**91.19%**	**91.64%**

Figure 1: Sample A habanero pepper % germination with H_2O by 12 labs
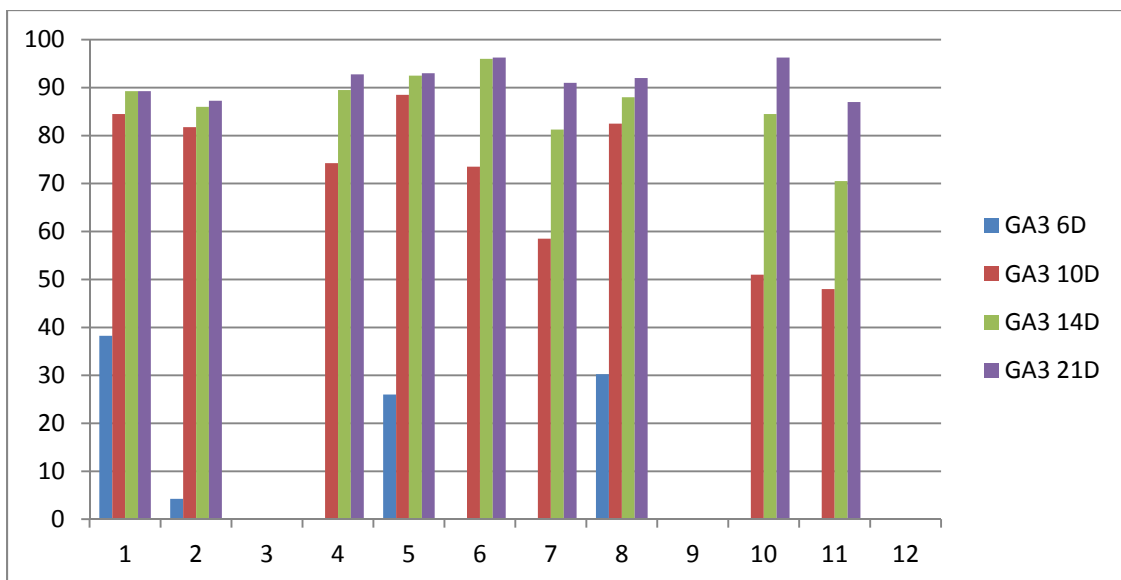
Figure 2: Sample A habanero pepper % germination with GA_3 by 9 labs

Sample B:

SAMPLE B: Average results for 12 laboratories		
Count	H_2O	GA_3
6 day	9.52%	8.17%
10 day	42.90%	45.69%
14 day	66.85%	67.17%
21 day	**80.04%**	**79.00%**

Figure 3: Sample B habanero pepper % germination with H_2O by 12 labs

Figure 4: Sample B habanero pepper % germination with GA_3 by 9 labs

DISCUSSION: There are several observations that can be made from the data received in this referee. Here is a brief summary:
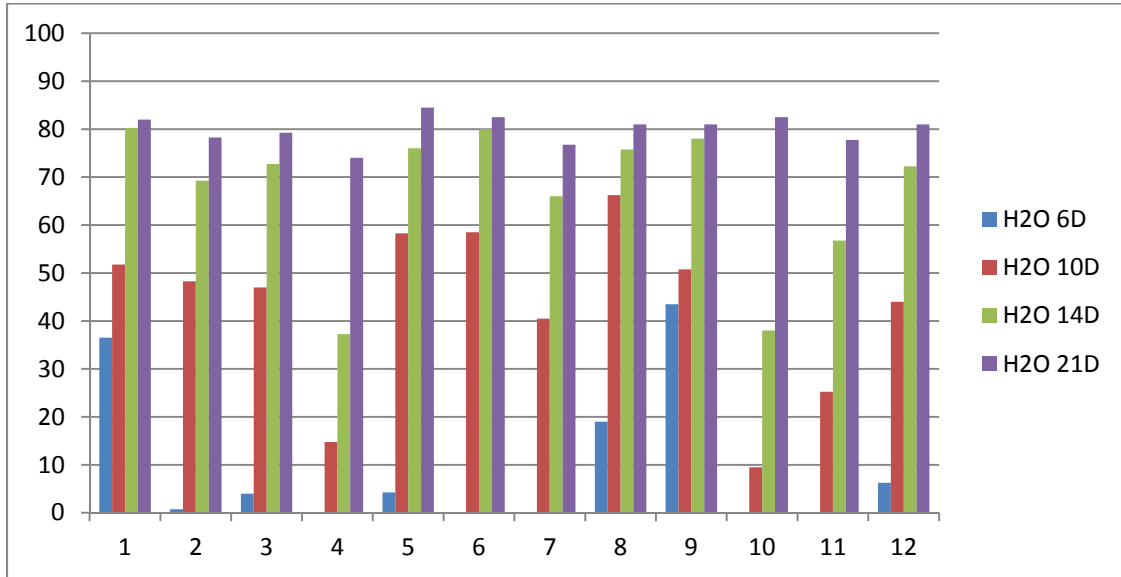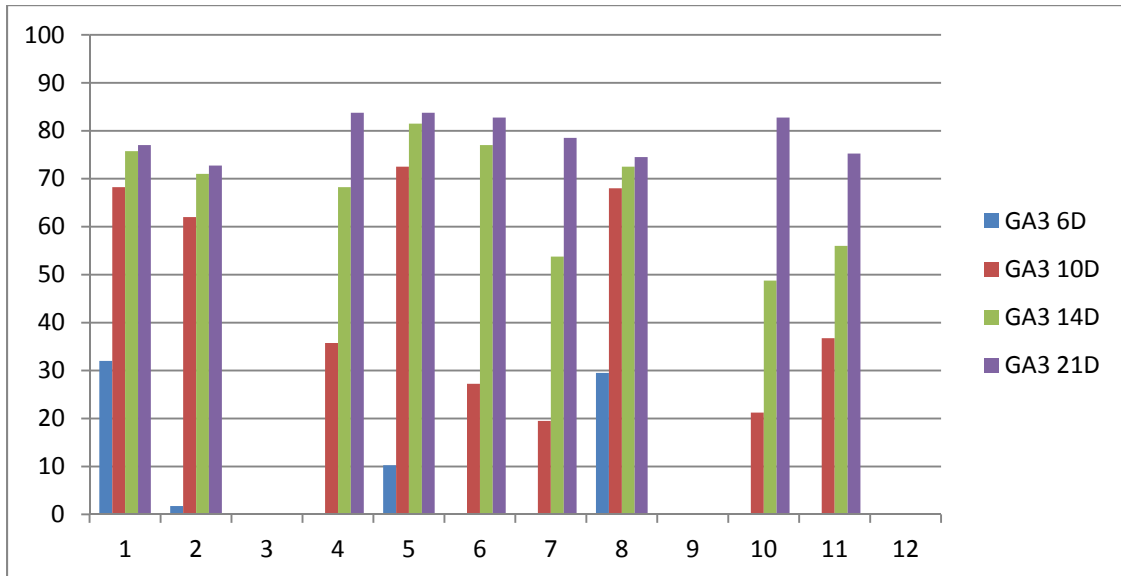
1. The average 6 day counts for both samples and test methods (H_2O and GA_3) were low as compared to the 10 day counts.
2. The average 21 day counts were significantly higher than the 14 day counts, indicating that at least for these two samples, optimum germination was not reached on 14 days.
3. GA_3 did not significantly affect the final test results, but it did seem to increase the initial germination rate slightly for some labs.
4. Most lab results were very consistent; no tests were out of tolerance for Sample B. For Sample A, one lab was out of tolerance using H_2O and two labs were out of tolerance using GA_3.

A more detailed statistical analysis of the data is presented in the accompanying document, Habanero Pepper Germination Referee 2012-2013 Southwest Region IV Statistical Comparisons.

CONCLUSIONS: The results of this referee support the objectives of changing the first count for habanero peppers (*Capsicum chinense*) from 6 days to 10 days, and changing the final count from 14 days to 21 days.

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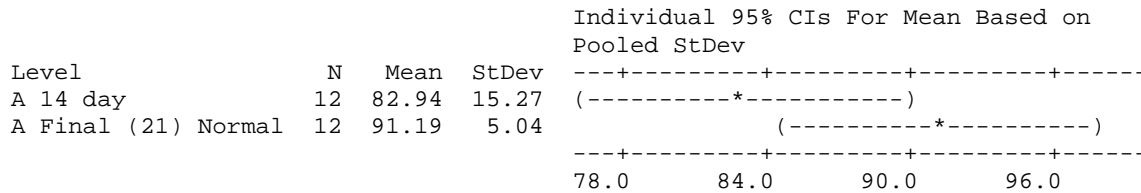# Habanero Pepper Germination Referee 2012-2013
Southwest Region IV

Statistical Comparisons:

Sample A (14 day germ vs 21 day germ)

One-way ANOVA: A 14 day, A Final (21) Normal

```
Source  DF    SS   MS     F      P
Factor   1   408  408  3.16  0.089
Error   22  2844  129
Total   23  3252

S = 11.37   R-Sq = 12.56%   R-Sq(adj) = 8.58%
```

```
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level                 N   Mean  StDev  ---+---------+---------+---------+------
A 14 day             12  82.94  15.27  (----------*-----------)
A Final (21) Normal  12  91.19   5.04              (----------*----------)
                                       ---+---------+---------+---------+------
                                       78.0      84.0      90.0      96.0

Pooled StDev = 11.37
```

```
Grouping Information Using Tukey Method

                      N   Mean  Grouping
A Final (21) Normal  12  91.19  A
A 14 day             12  82.94  A

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: A 14 day, A Final (21) Normal

```
Two-sample T for A 14 day vs A Final (21) Normal

                      N   Mean  StDev  SE Mean
A 14 day             12   82.9   15.3      4.4
A Final (21) Normal  12  91.19   5.04      1.5


Difference = mu (A 14 day) - mu (A Final (21) Normal)
Estimate for difference:  -8.25
95% CI for difference:  (-18.28, 1.78)
T-Test of difference = 0 (vs not =): T-Value = -1.78  P-Value = 0.099  DF = 13
```
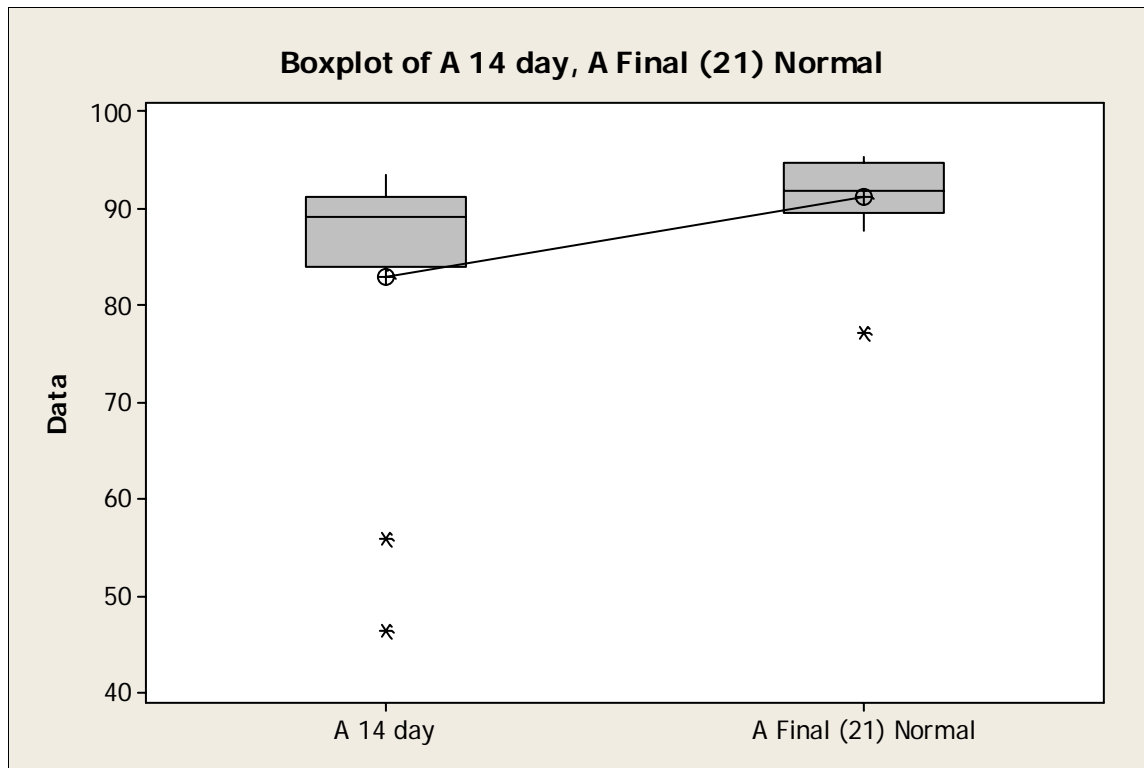
Boxplot of A 14 day, A Final (21) Normal

Discussion: The p-value of 0.099 indicates that the two means are not significantly different, however the standard deviation or 15.27 among labs for the 14 day count would be unacceptably high.

Sample A with GA$_3$ (14 day germ vs 21 day germ)

One-way ANOVA: A GA 14 Day, A GA Final(21) Normal

```
Source  DF    SS     MS     F      P
Factor   1  124.0  124.0  3.78  0.070
Error   16  525.4   32.8
Total   17  649.4

S = 5.730   R-Sq = 19.10%   R-Sq(adj) = 14.04%


Level                    N    Mean   StDev
A GA 14 Day              9  86.389  7.355
A GA Final(21) Normal    9  91.639  3.403

                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level                    -----+---------+---------+---------+----
A GA 14 Day              (-----------*----------)
A GA Final(21) Normal                (-----------*----------)
                         -----+---------+---------+---------+----
                           84.0      87.5      91.0      94.5

Pooled StDev = 5.730
```
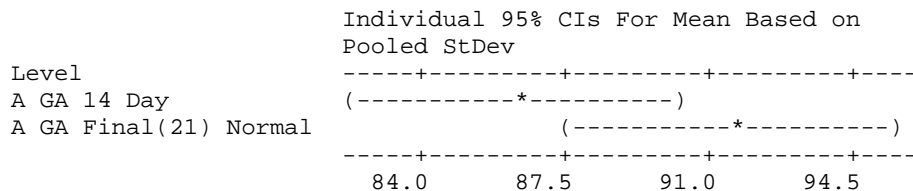
```
Grouping Information Using Tukey Method

                       N    Mean  Grouping
A GA Final(21) Normal  9  91.639  A
A GA 14 Day            9  86.389  A

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: A GA 14 Day, A GA Final(21) Normal

```
Two-sample T for A GA 14 Day vs A GA Final(21) Normal

                       N   Mean  StDev  SE Mean
A GA 14 Day            9  86.39   7.35      2.5
A GA Final(21) Normal  9  91.64   3.40      1.1


Difference = mu (A GA 14 Day) - mu (A GA Final(21) Normal)
Estimate for difference:  -5.25
95% CI for difference:  (-11.20, 0.70)
T-Test of difference = 0 (vs not =): T-Value = -1.94   P-Value = 0.078   DF = 11
```


Boxplot of A GA 14 Day, A GA Final(21) Normal

Discussion: The p-value of 0.078 indicates that the means are not significantly different, however, again the standard deviation among labs is high (7.35) at the 14 day count.

Sample B (14 day germ vs 21 day germ):

One-way ANOVA: B 14 Day, B Final (21) Normal

```
Source  DF    SS    MS     F      P
Factor   1  1043  1043  8.78  0.007
Error   22  2616   119
Total   23  3659

S = 10.90   R-Sq = 28.52%   R-Sq(adj) = 25.27%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level                  N   Mean  StDev  ----+---------+---------+---------+-----
B 14 Day              12  66.85  15.14  (---------*--------)
B Final (21) Normal   12  80.04   2.94                   (--------*---------)
                                        ----+---------+---------+---------+-----
                                          63.0      70.0      77.0      84.0

Pooled StDev = 10.90


Grouping Information Using Tukey Method

                       N   Mean  Grouping
B Final (21) Normal   12  80.04  A
B 14 Day              12  66.85     B

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: B 14 Day, B Final (21) Normal

```
Two-sample T for B 14 Day vs B Final (21) Normal

                       N   Mean  StDev  SE Mean
B 14 Day              12   66.9   15.1      4.4
B Final (21) Normal   12  80.04   2.94     0.85


Difference = mu (B 14 Day) - mu (B Final (21) Normal)
Estimate for difference:  -13.19
95% CI for difference:  (-22.99, -3.39)
T-Test of difference = 0 (vs not =): T-Value = -2.96   P-Value = 0.013   DF = 11
```
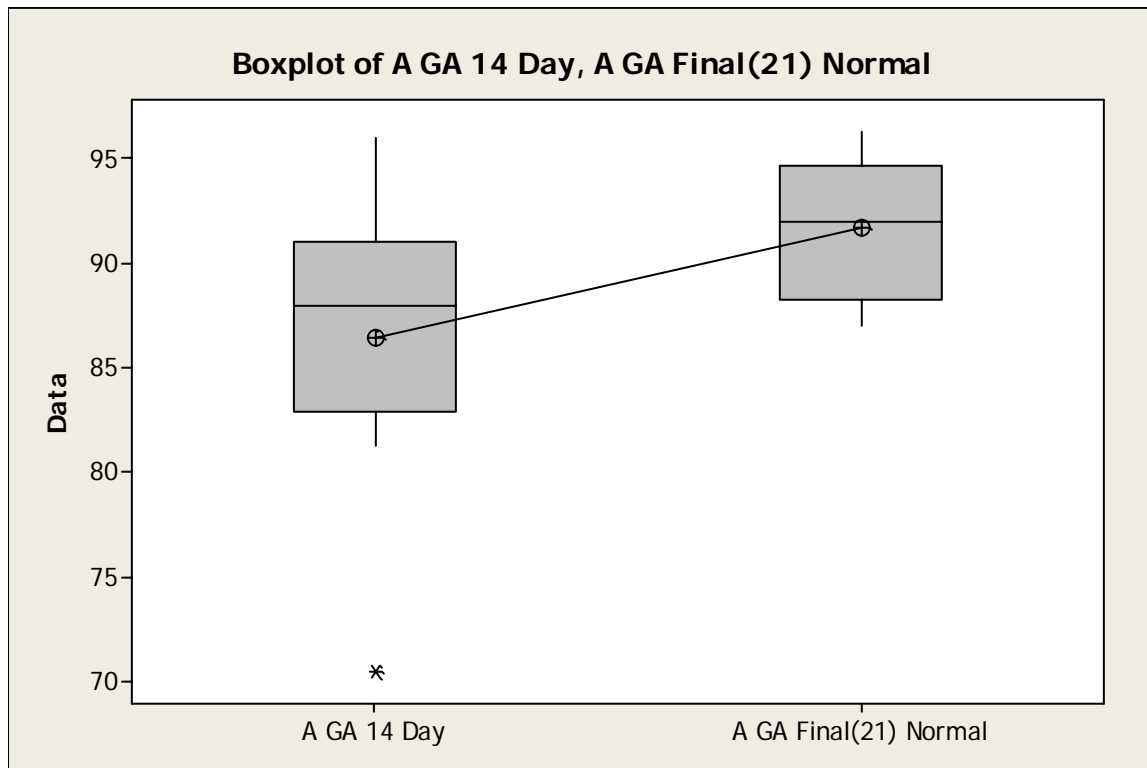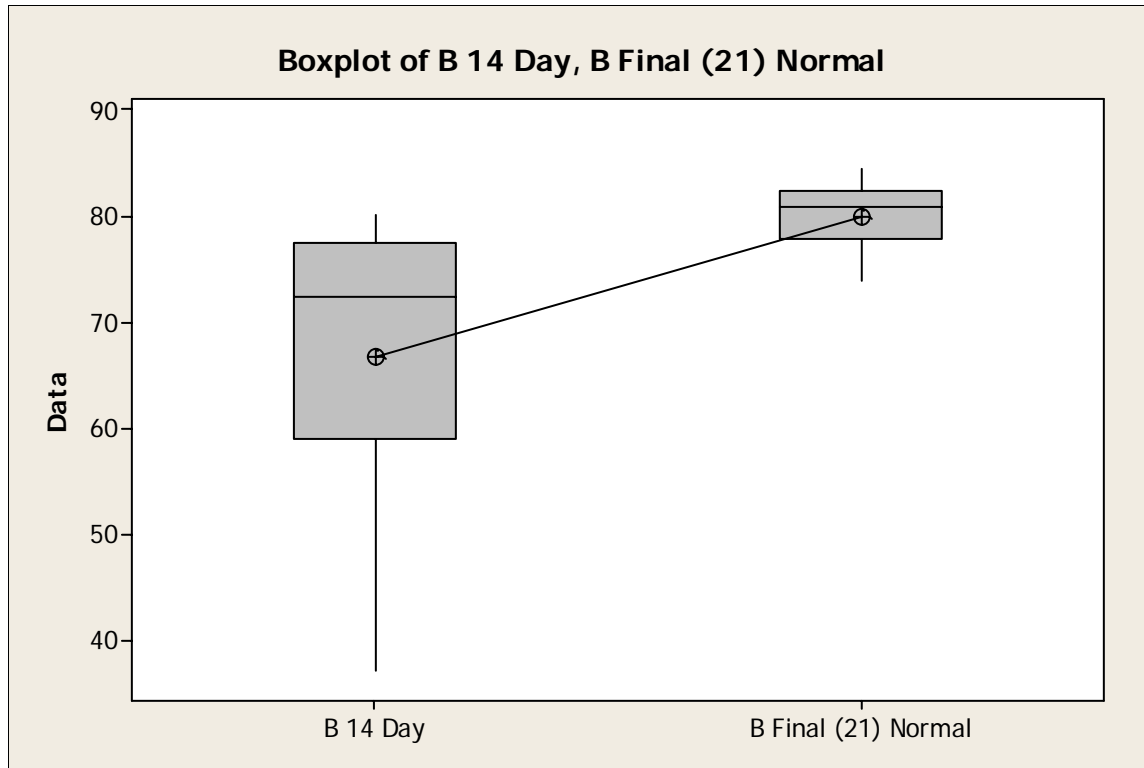
Boxplot of B 14 Day, B Final (21) Normal

Discussion: The p-value of 0.013 indicates that the two means are significantly different.
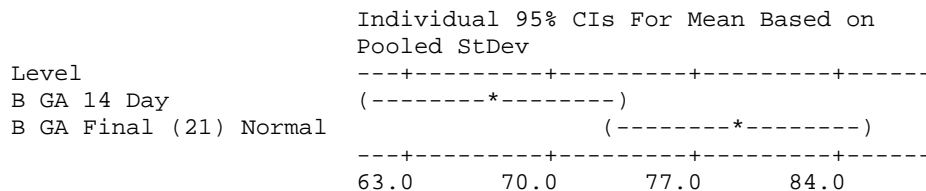

Sample B with GA₃ (14 day germ vs 21 day germ)

One-way ANOVA: B GA 14 Day, B GA Final (21) Normal

```
Source  DF      SS     MS     F      P
Factor   1   630.1  630.1  8.30  0.011
Error   16  1214.9   75.9
Total   17  1845.0

S = 8.714   R-Sq = 34.15%   R-Sq(adj) = 30.04%
```

```
Level                  N    Mean   StDev
B GA 14 Day            9  67.167  11.533
B GA Final (21) Normal 9  79.000   4.343

                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level                  ---+---------+---------+---------+------
B GA 14 Day            (--------*--------)
B GA Final (21) Normal                 (--------*--------)
                       ---+---------+---------+---------+------
                       63.0      70.0      77.0      84.0

Pooled StDev = 8.714
```

```
Grouping Information Using Tukey Method

                        N    Mean   Grouping
B GA Final (21) Normal  9   79.000  A
B GA 14 Day             9   67.167     B

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: B GA 14 Day, B GA Final (21) Normal

```
Two-sample T for B GA 14 Day vs B GA Final (21) Normal

                        N    Mean   StDev  SE Mean
B GA 14 Day             9    67.2   11.5     3.8
B GA Final (21) Normal  9   79.00   4.34     1.4


Difference = mu (B GA 14 Day) - mu (B GA Final (21) Normal)
Estimate for difference:  -11.83
95% CI for difference:  (-20.99, -2.68)
T-Test of difference = 0 (vs not =): T-Value = -2.88   P-Value = 0.016   DF = 10
```
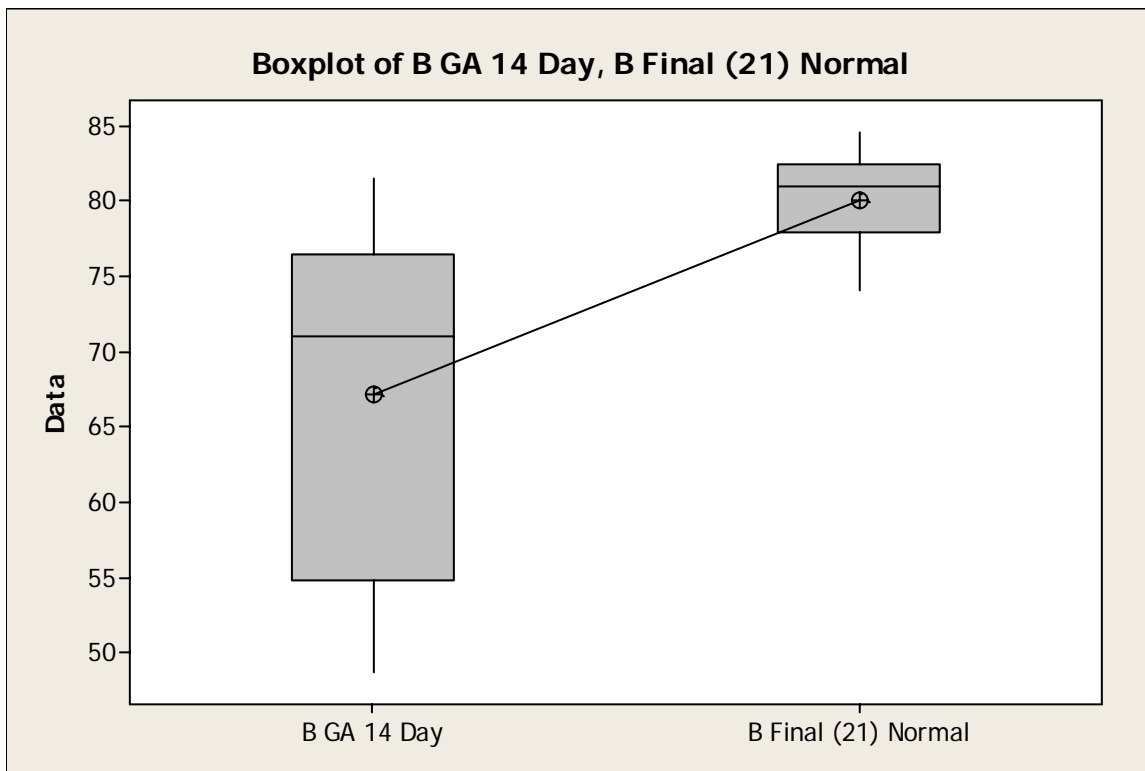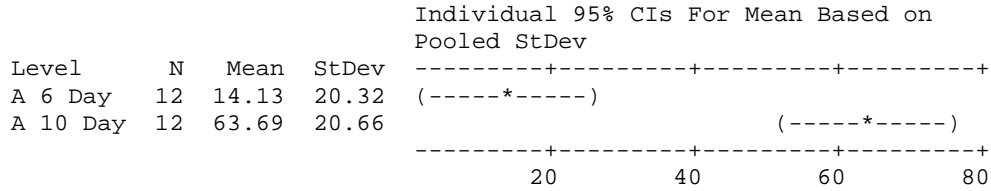

Boxplot of B GA 14 Day, B Final (21) Normal

Discussion: The p-value of 0.016 indicates that the two means are significantly different.

Sample A (6 day germ vs 10 day germ)

One-way ANOVA: A 6 Day, A 10 Day

```
Source   DF      SS      MS      F       P
Factor    1   14739   14739   35.10   0.000
Error    22    9238     420
Total    23   23977

S = 20.49   R-Sq = 61.47%   R-Sq(adj) = 59.72%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level       N   Mean   StDev  ---------+---------+---------+---------+
A 6 Day    12  14.13   20.32  (-----*-----)
A 10 Day   12  63.69   20.66                           (-----*-----)
                              ---------+---------+---------+---------+
                                      20        40        60        80


Pooled StDev = 20.49



Grouping Information Using Tukey Method

            N   Mean   Grouping
A 10 Day   12  63.69   A
A 6 Day    12  14.13      B

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: A 6 Day, A 10 Day

```
Two-sample T for A 6 Day vs A 10 Day

            N   Mean   StDev   SE Mean
A 6 Day    12  14.1    20.3      5.9
A 10 Day   12  63.7    20.7      6.0


Difference = mu (A 6 Day) - mu (A 10 Day)
Estimate for difference:  -49.56
95% CI for difference:  (-66.96, -32.16)
T-Test of difference = 0 (vs not =): T-Value = -5.92  P-Value = 0.000  DF = 21
```
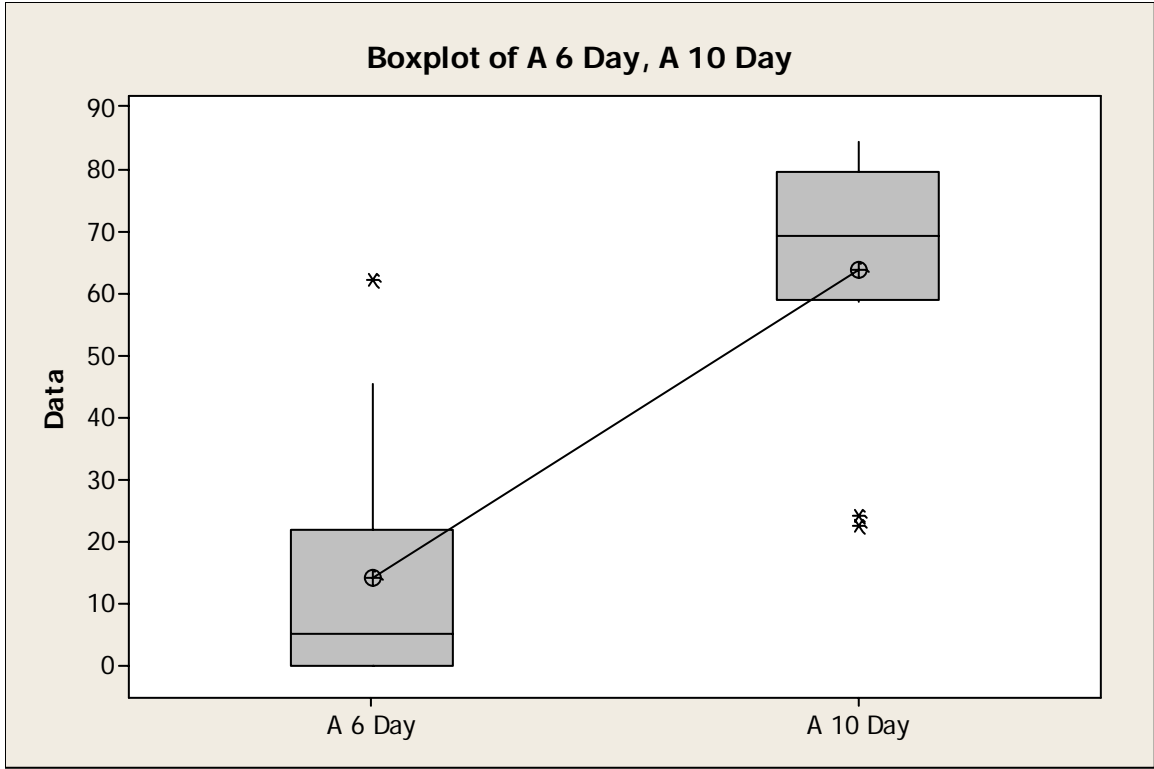
Boxplot of A 6 Day, A 10 Day

Discussion: The p-value of 0.000 indicates that the two means are significantly different.

Sample A with GA$_3$ (6 day germ vs 10 day germ)

One-way ANOVA: A GA 6 Day, A GA 10 day

```
Source  DF      SS      MS      F      P
Factor   1   14649   14649  59.09  0.000
Error   16    3967     248
Total   17   18616

S = 15.75   R-Sq = 78.69%   R-Sq(adj) = 77.36%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level          N   Mean   StDev   +---------+---------+---------+---------
A GA 6 Day     9  10.97   15.77   (----*-----)
A GA 10 day    9  68.03   15.72                             (-----*-----)
                                   +---------+---------+---------+---------
                                   0        20        40        60

Pooled StDev = 15.75


Grouping Information Using Tukey Method

             N   Mean   Grouping
A GA 10 day  9  68.03   A
A GA 6 Day   9  10.97      B
```

Means that do not share a letter are significantly different.

Two-Sample T-Test and CI: A GA 6 Day, A GA 10 day

```
Two-sample T for A GA 6 Day vs A GA 10 day

             N   Mean  StDev  SE Mean
A GA 6 Day   9   11.0   15.8      5.3
A GA 10 day  9   68.0   15.7      5.2


Difference = mu (A GA 6 Day) - mu (A GA 10 day)
Estimate for difference:  -57.06
95% CI for difference:  (-72.88, -41.23)
T-Test of difference = 0 (vs not =): T-Value = -7.69  P-Value = 0.000  DF = 15
```
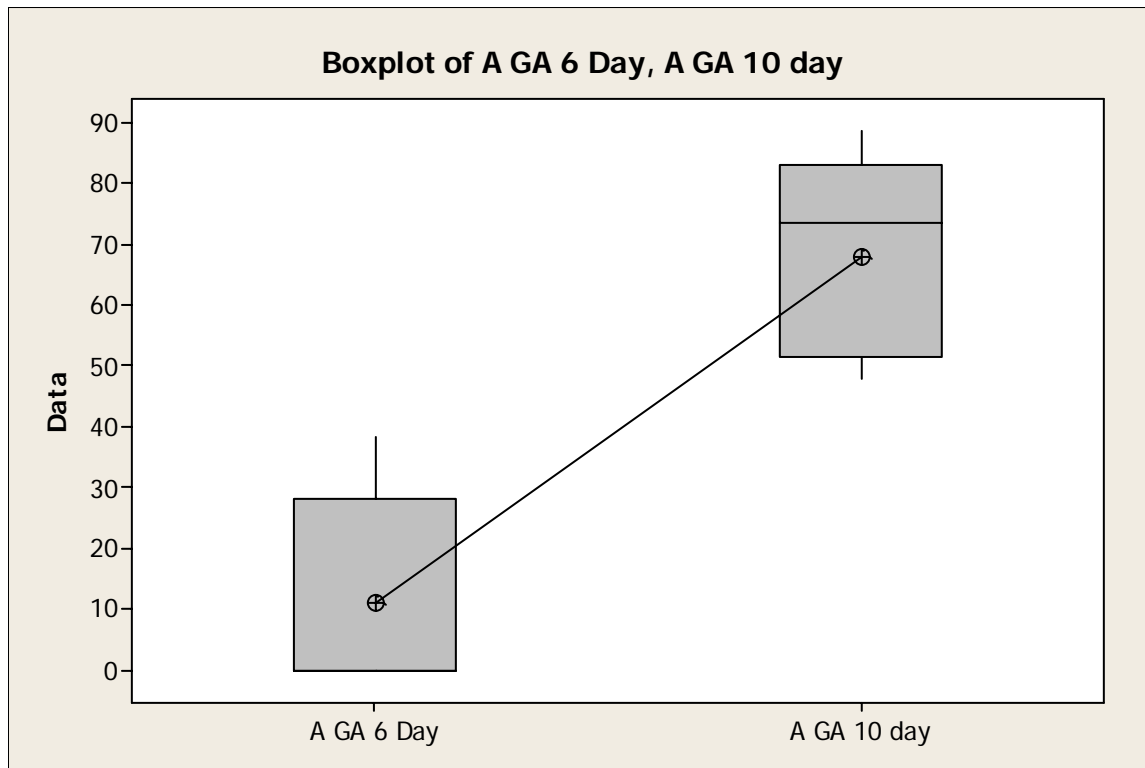

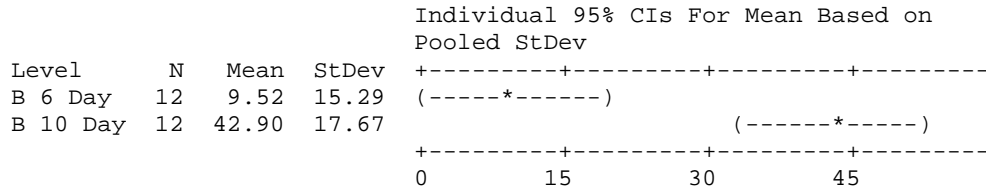
Boxplot of A GA 6 Day, A GA 10 day

Discussion: The p-value of 0.000 indicates that the two means are significantly different.

Sample B (6 day germ vs 10 day germ)

One-way ANOVA: B 6 Day, B 10 Day

```
Source  DF     SS    MS      F      P
Factor   1   6683  6683  24.48  0.000
Error   22   6007   273
Total   23  12691
```

```
S = 16.52   R-Sq = 52.66%   R-Sq(adj) = 50.51%


                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level       N   Mean  StDev  +---------+---------+---------+---------
B 6 Day    12   9.52  15.29  (-----*------)
B 10 Day   12  42.90  17.67                     (------*-----)
                             +---------+---------+---------+---------
                             0        15        30        45


Pooled StDev = 16.52


Grouping Information Using Tukey Method

            N   Mean  Grouping
B 10 Day   12  42.90  A
B 6 Day    12   9.52     B

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: B 6 Day, B 10 Day

```
Two-sample T for B 6 Day vs B 10 Day

            N  Mean  StDev  SE Mean
B 6 Day    12   9.5  15.3      4.4
B 10 Day   12  42.9  17.7      5.1


Difference = mu (B 6 Day) - mu (B 10 Day)
Estimate for difference:  -33.38
95% CI for difference:  (-47.40, -19.35)
T-Test of difference = 0 (vs not =): T-Value = -4.95  P-Value = 0.000  DF = 21
```
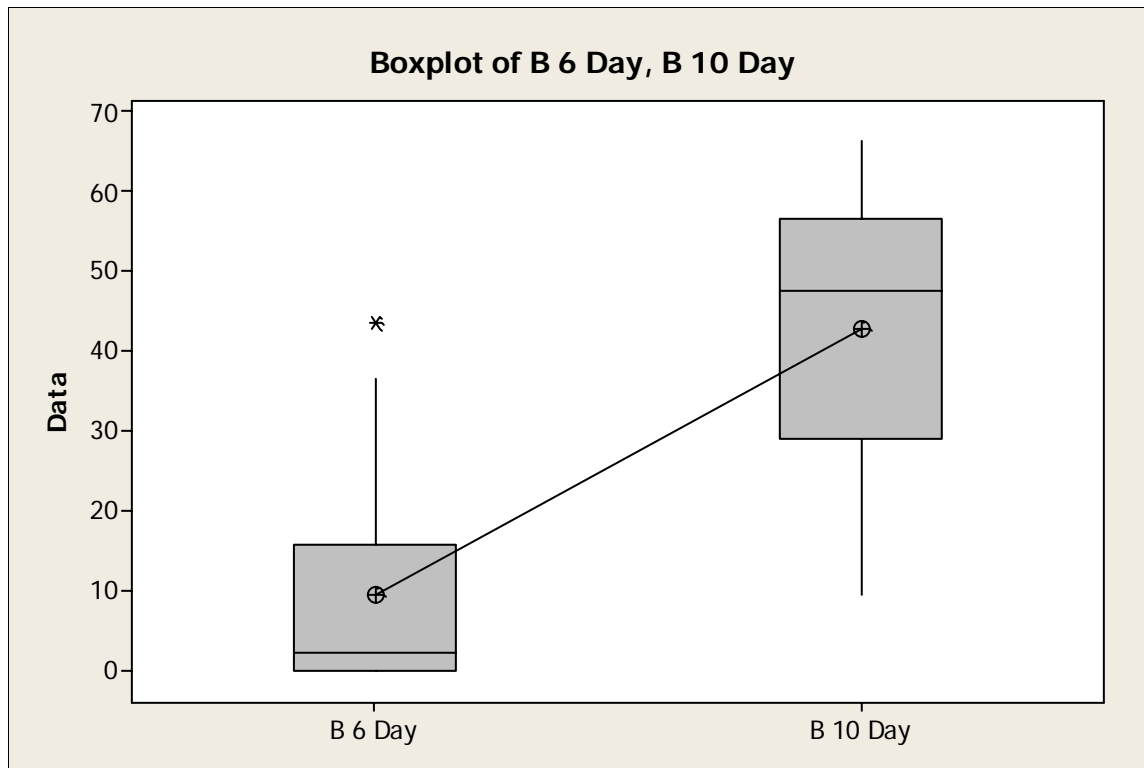
Boxplot of B 6 Day, B 10 Day

Discussion: The p-value of 0.000 indicates that the two means are significantly different.

Sample B with GA$_3$ (6 day germ vs 10 day germ)

One-way ANOVA: B GA 6 Day, B GA 10 Day

```
Source   DF      SS     MS      F       P
Factor    1    6338   6338   19.52   0.000
Error    16    5196    325
Total    17   11533

S = 18.02   R-Sq = 54.95%   R-Sq(adj) = 52.13%


                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level          N   Mean  StDev   ---+---------+---------+---------+------
B GA 6 Day     9   8.17  13.24   (-------*-------)
B GA 10 Day    9  45.69  21.78                        (-------*-------)
                               ---+---------+---------+---------+------
                                  0        16        32        48

Pooled StDev = 18.02


Grouping Information Using Tukey Method

              N   Mean  Grouping
B GA 10 Day   9  45.69  A
B GA 6 Day    9   8.17     B
```

Means that do not share a letter are significantly different.

Two-Sample T-Test and CI: B GA 6 Day, B GA 10 Day

```
Two-sample T for B GA 6 Day vs B GA 10 Day

             N   Mean   StDev   SE Mean
B GA 6 Day   9    8.2    13.2       4.4
B GA 10 Day  9   45.7    21.8       7.3


Difference = mu (B GA 6 Day) - mu (B GA 10 Day)
Estimate for difference:  -37.53
95% CI for difference:  (-55.88, -19.18)
T-Test of difference = 0 (vs not =): T-Value = -4.42   P-Value = 0.001   DF = 13
```
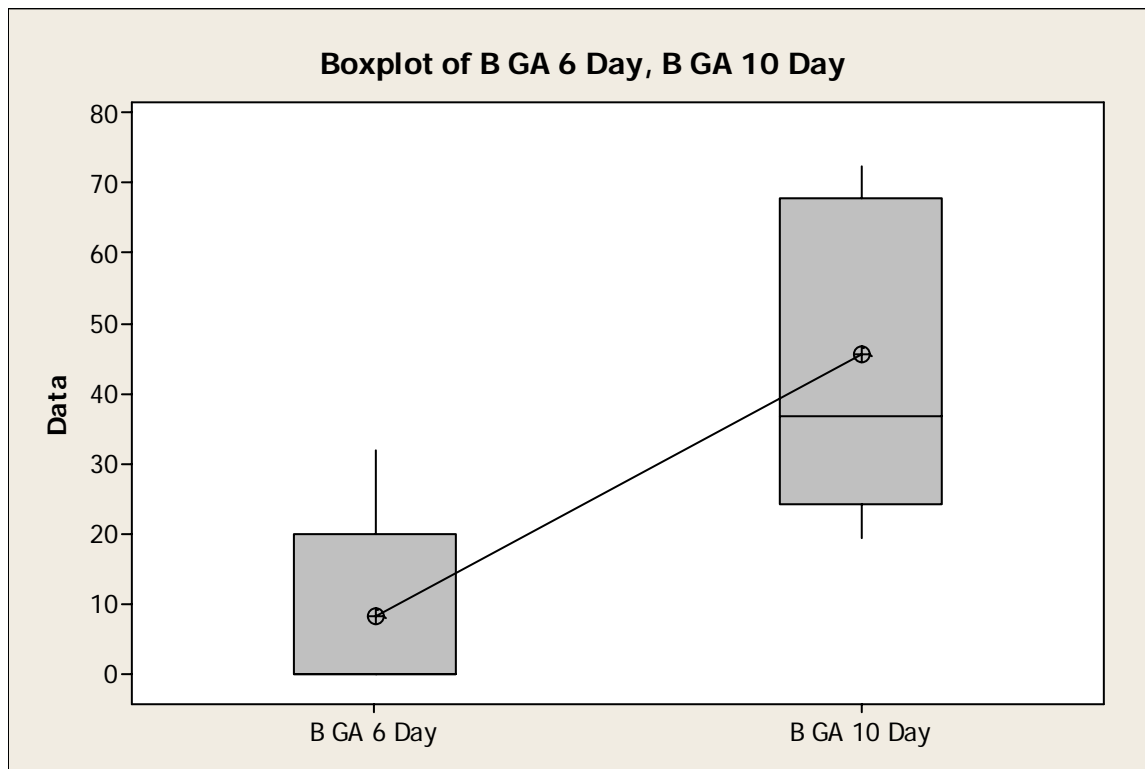

Boxplot of B GA 6 Day, B GA 10 Day

Discussion: The p-value of 0.001 indicates that the two means are significantly different.

Sample A with H_2O vs A with GA_3 (21 day germ)
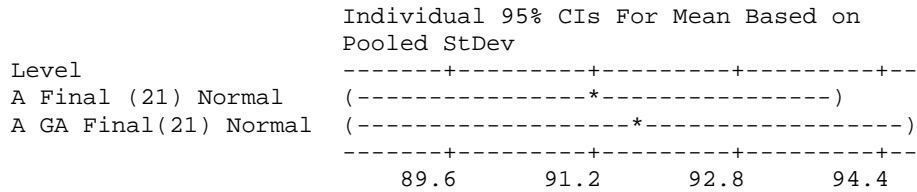
One-way ANOVA: A Final (21) Normal, A GA Final(21) Normal

```
Source  DF    SS    MS     F      P
Factor   1   1.0   1.0   0.05   0.819
```

```
Error   19  371.8  19.6
Total   20  372.8

S = 4.423   R-Sq = 0.28%   R-Sq(adj) = 0.00%




Level                  N    Mean   StDev
A Final (21) Normal    12  91.188  5.038
A GA Final(21) Normal   9  91.639  3.403

                       Individual 95% CIs For Mean Based on
                       Pooled StDev
Level                  -------+---------+---------+---------+--
A Final (21) Normal    (---------------*----------------)
A GA Final(21) Normal  (------------------*------------------)
                       -------+---------+---------+---------+--
                          89.6      91.2      92.8      94.4


Pooled StDev = 4.423



Grouping Information Using Tukey Method

                       N    Mean  Grouping
A GA Final(21) Normal   9  91.639  A
A Final (21) Normal    12  91.188  A

Means that do not share a letter are significantly different.
```
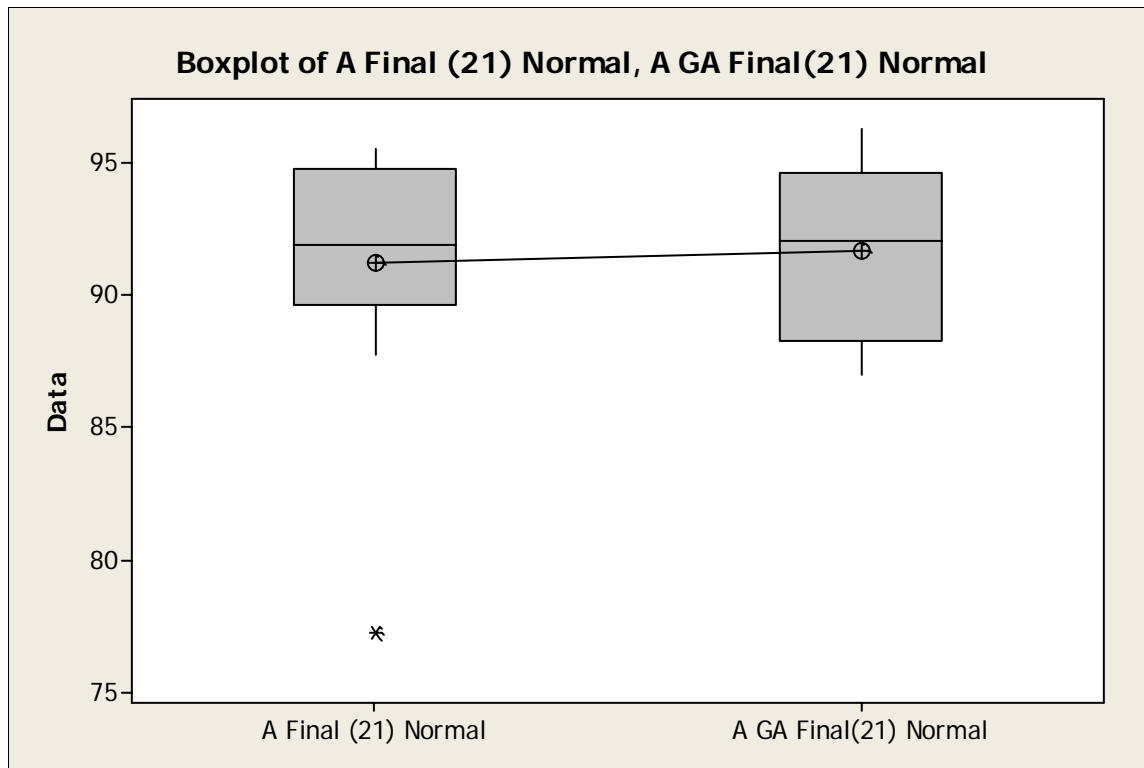
Two-Sample T-Test and CI: A Final (21) Normal, A GA Final(21) Normal

```
Two-sample T for A Final (21) Normal vs A GA Final(21) Normal

                       N    Mean  StDev  SE Mean
A Final (21) Normal    12  91.19   5.04      1.5
A GA Final(21) Normal   9  91.64   3.40      1.1


Difference = mu (A Final (21) Normal) - mu (A GA Final(21) Normal)
Estimate for difference:  -0.45
95% CI for difference:  (-4.33, 3.42)
T-Test of difference = 0 (vs not =): T-Value = -0.24  P-Value = 0.809  DF = 18
```

Boxplot of A Final (21) Normal, A GA Final(21) Normal

Discussion: the p-value of 0.809 indicates there is not a significant difference in the means of the two treatments.

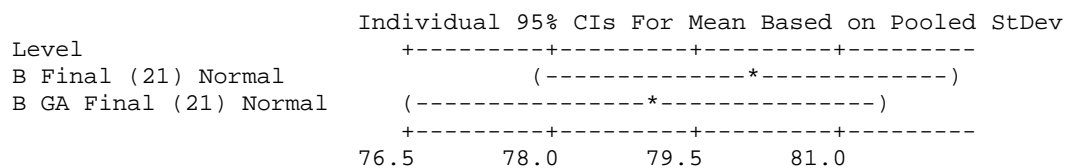**Sample B with H_2O vs B with GA_3 (21 day germ)**

One-way ANOVA: B Final (21) Normal, B GA Final (21) Normal

```
Source  DF     SS    MS     F      P
Factor   1    5.6   5.6  0.43  0.519
Error   19  245.9  12.9
Total   20  251.4

S = 3.597   R-Sq = 2.22%   R-Sq(adj) = 0.00%


Level                    N    Mean   StDev
B Final (21) Normal     12  80.042   2.938
B GA Final (21) Normal   9  79.000   4.343

                         Individual 95% CIs For Mean Based on Pooled StDev
Level                      +---------+---------+---------+---------
B Final (21) Normal                    (--------------*-------------)
B GA Final (21) Normal       (----------------*---------------)
                           +---------+---------+---------+---------
                         76.5      78.0      79.5      81.0

Pooled StDev = 3.597


Grouping Information Using Tukey Method
```

```
                          N    Mean  Grouping
B Final (21) Normal       12  80.042  A
B GA Final (21) Normal     9  79.000  A

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: B Final (21) Normal, B GA Final (21) Normal

```
Two-sample T for B Final (21) Normal vs B GA Final (21) Normal

                          N   Mean  StDev  SE Mean
B Final (21) Normal       12  80.04   2.94    0.85
B GA Final (21) Normal     9  79.00   4.34     1.4


Difference = mu (B Final (21) Normal) - mu (B GA Final (21) Normal)
Estimate for difference:  1.04
95% CI for difference:  (-2.58, 4.67)
T-Test of difference = 0 (vs not =): T-Value = 0.62  P-Value = 0.545  DF = 13
```
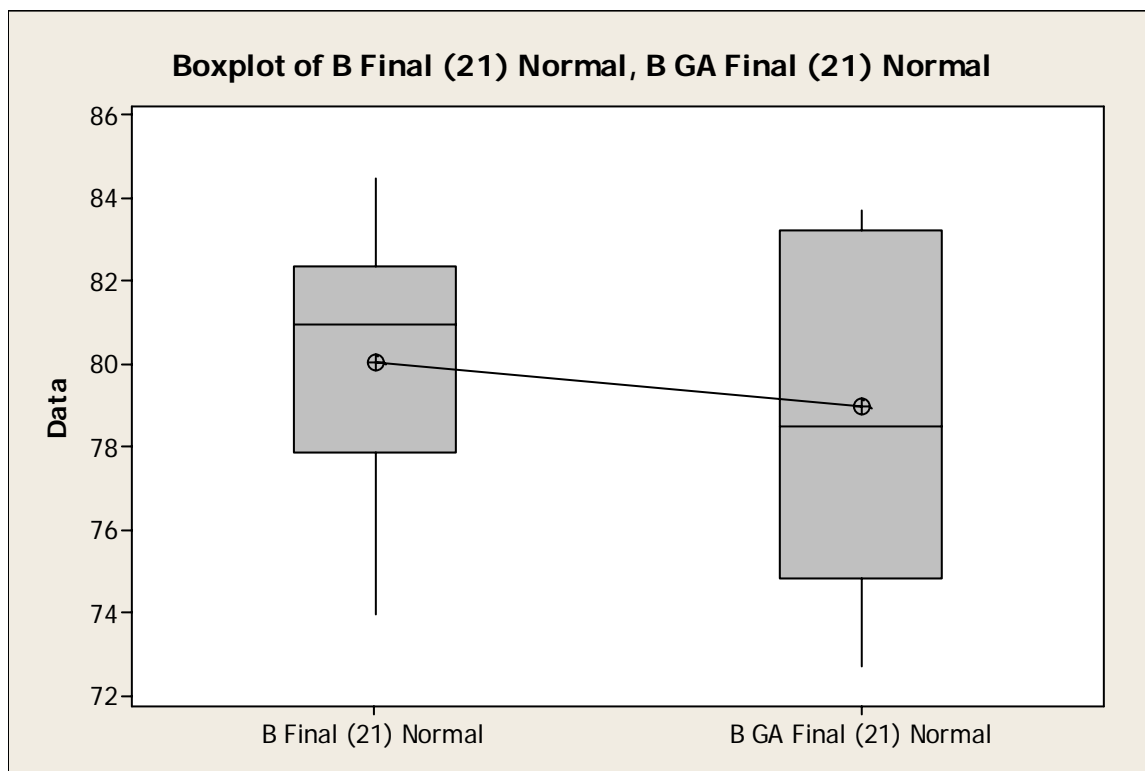

Boxplot of B Final (21) Normal, B GA Final (21) Normal

Discussion: the p-value of 0.809 indicates there is not a significant difference in the means of the two treatments.