CPRAB 2019 Greenhouse Experiment Data Analysis

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Experimental Background

- Experiment Duration: 120 Days in Greenhouse as a Randomized Complete Block Design
- 5 Randomized Complete Blocks
- 2 Levels of Nitrogen Fertilization (10% and 100% Hoagland Complete Fertilizer Nitrogen)
  - Fertilized irrigation delivered daily at 500 mL per plant.
- 5 Inoculation Levels
  - Mock-inoculated
  - Microbial Pool Inoculation
  - Isolate 201900 Inoculation
  - Isolate 201849 Inoculation
  - Isolate 200810 Inoculation
- Number of Inoculation events: 3
- Inoculated first in the laboratory vial during rooting process
- Inoculated a second time upon transplant to greenhouse pot
- Inoculated a third time 2-weeks post greenhouse transplantation
- Soil Mixture:
- Calcined Clay
- Coconut Coire
- Sand
  - Ratio: 2:3:1 (Sand:Coconut Coire: Calcined Clay)
- Response Variables:
- Dry Shoot Weight
- Dry Root Weight
- Fresh Tuber Weight
- Tuber Number

Data Analysis

Full DataSet

Linear Modeling

Data measurements were fit to a linear model using the `lm` function of R. The normality of the data was assessed by visualizing parameters of each linear model in two ways:

- Normal Q-Q plots (Standardized residuals vs. Theoretical Quantiles)
- Scale - Location plots (Squareroot of Standard residuals vs. fitted values)

Create Stats Functions for Bar Plots

Stats functions created for standard error of mean and 95% confidence intervals

High Nitrogen and Low Nitrogen treated plants had clear differences in total biomass accumulation. Comparisons between these two groups are not made in this report.

High Nitrogen Treatment

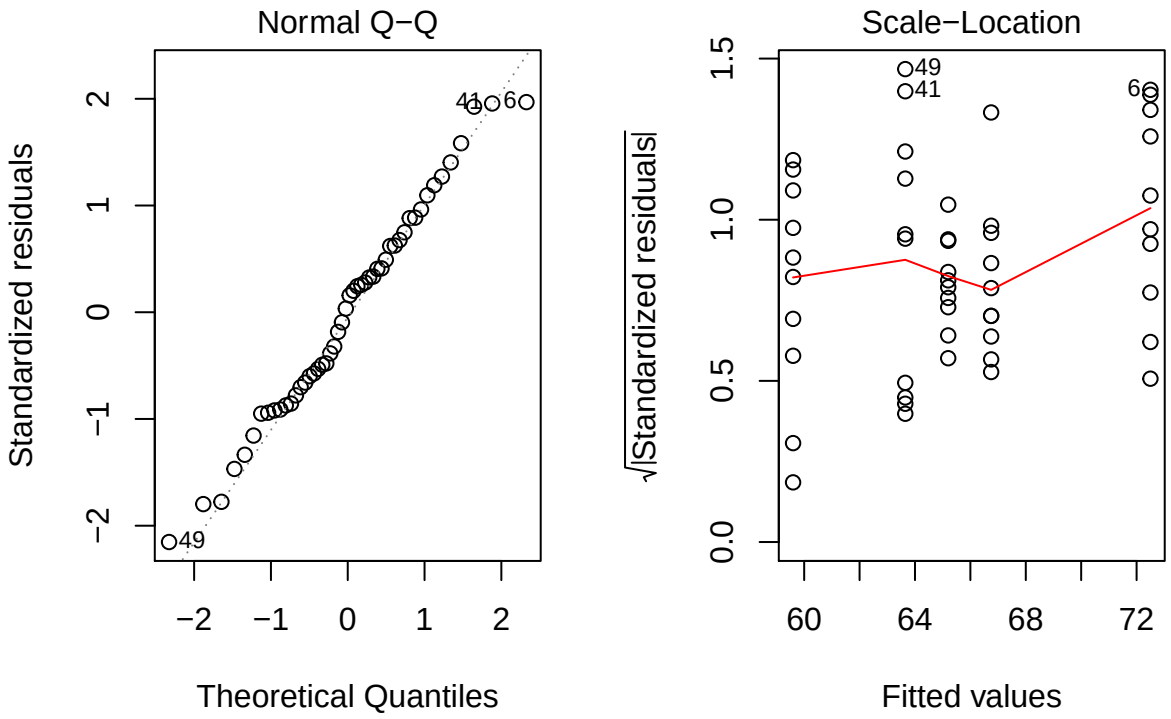
```
## Classes 'spec_tbl_df', 'tbl_df', 'tbl' and 'data.frame': 50 obs. of  12 variables:
##  $ sample          : chr  "A-N1-1.1" "A-N1-1.2" "A-N1-2.1" "A-N1-2.2" ...
##  $ nitrogen        : chr  "N1" "N1" "N1" "N1" ...
##  $ inoc            : chr  "Mock" "Mock" "Mock" "Mock" ...
##  $ trt             : Factor w/ 5 levels "A","B","C","D",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ plot            : Factor w/ 5 levels "R1","R2","R3",..: 1 1 2 2 3 3 4 4 5 5 ...
##  $ rep             : Factor w/ 2 levels "1","2": 1 2 1 2 1 2 1 2 1 2 ...
##  $ n_tuber         : num  6 6 5 4 4 9 23 8 13 12 ...
##  $ tuber_wt_g      : num  139 202 289 257 312 ...
##  $ shoot_dry_wt_bag: num  105.5 118.5 104.5 102 94.5 ...
##  $ shoot_dry_wt_g  : num  62.5 75.5 61.5 59 51.5 95.5 65.5 68 95 91 ...
##  $ root_dry_wt_g   : num  13.5 14.5 23 36.5 23 66.5 35 23.5 43.5 37.5 ...
##  $ comments        : chr  NA NA NA NA ...
##  - attr(*, "spec")=
##   .. cols(
##   ..   sample = col_character(),
##   ..   nitrogen = col_character(),
##   ..   inoc = col_character(),
##   ..   trt = col_factor(levels = NULL, ordered = FALSE, include_na = FALSE),
##   ..   plot = col_factor(levels = NULL, ordered = FALSE, include_na = FALSE),
##   ..   rep = col_factor(levels = NULL, ordered = FALSE, include_na = FALSE),
##   ..   n_tuber = col_number(),
##   ..   tuber_wt_g = col_number(),
##   ..   shoot_dry_wt_bag = col_number(),
##   ..   shoot_dry_wt_g = col_number(),
##   ..   root_dry_wt_g = col_number(),
##   ..   comments = col_character()
##   .. )
```

```
##     sample            nitrogen            inoc           trt    plot
##  Length:50          Length:50          Length:50          A:10   R1:10
##  Class :character   Class :character   Class :character   B:10   R2:10
##  Mode  :character   Mode  :character   Mode  :character   C:10   R3:10
##                                                           D:10   R4:10
##                                                           E:10   R5:10
##
##  rep       n_tuber        tuber_wt_g    shoot_dry_wt_bag shoot_dry_wt_g
##  1:25   Min.   : 4.00   Min.   :139.0   Min.   : 81.5    Min.   :38.50
##  2:25   1st Qu.: 6.00   1st Qu.:207.0   1st Qu.:100.8    1st Qu.:57.75
##         Median : 8.50   Median :292.5   Median :108.5    Median :65.50
##         Mean   :10.14   Mean   :300.9   Mean   :108.5    Mean   :65.54
##         3rd Qu.:12.00   3rd Qu.:389.2   3rd Qu.:116.9    3rd Qu.:73.88
##         Max.   :32.00   Max.   :524.0   Max.   :138.5    Max.   :95.50
```

```
##  root_dry_wt_g     comments
##  Min.   :10.00   Length:50
##  1st Qu.:23.00   Class :character
##  Median :37.00   Mode  :character
##  Mean   :36.70
##  3rd Qu.:45.12
##  Max.   :81.00
```

Shoot Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: shoot_dry_wt_g
##           Df Sum Sq Mean Sq F value Pr(>F)
## inoc       4  888.8  222.19  1.4654 0.2285
## Residuals 45 6823.1  151.63
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

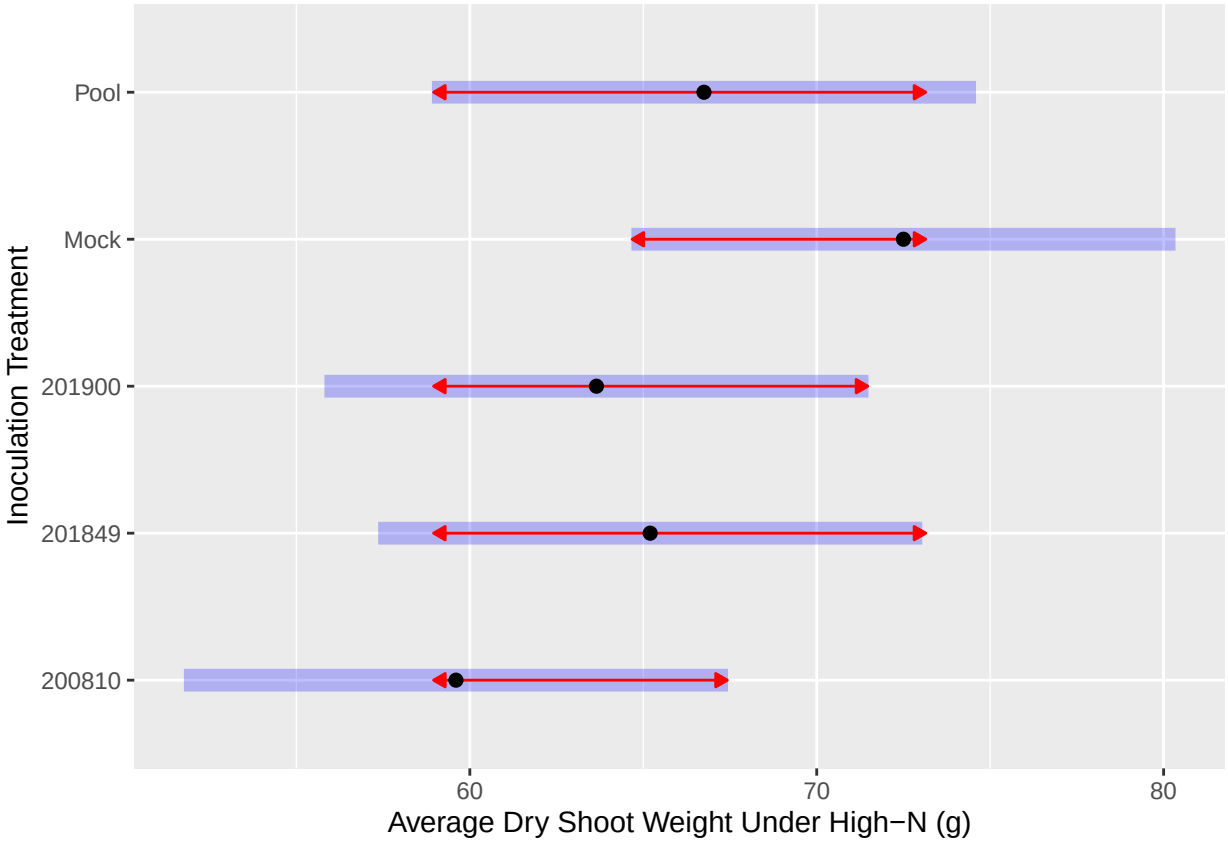
Tukey method of correction for multiple testing

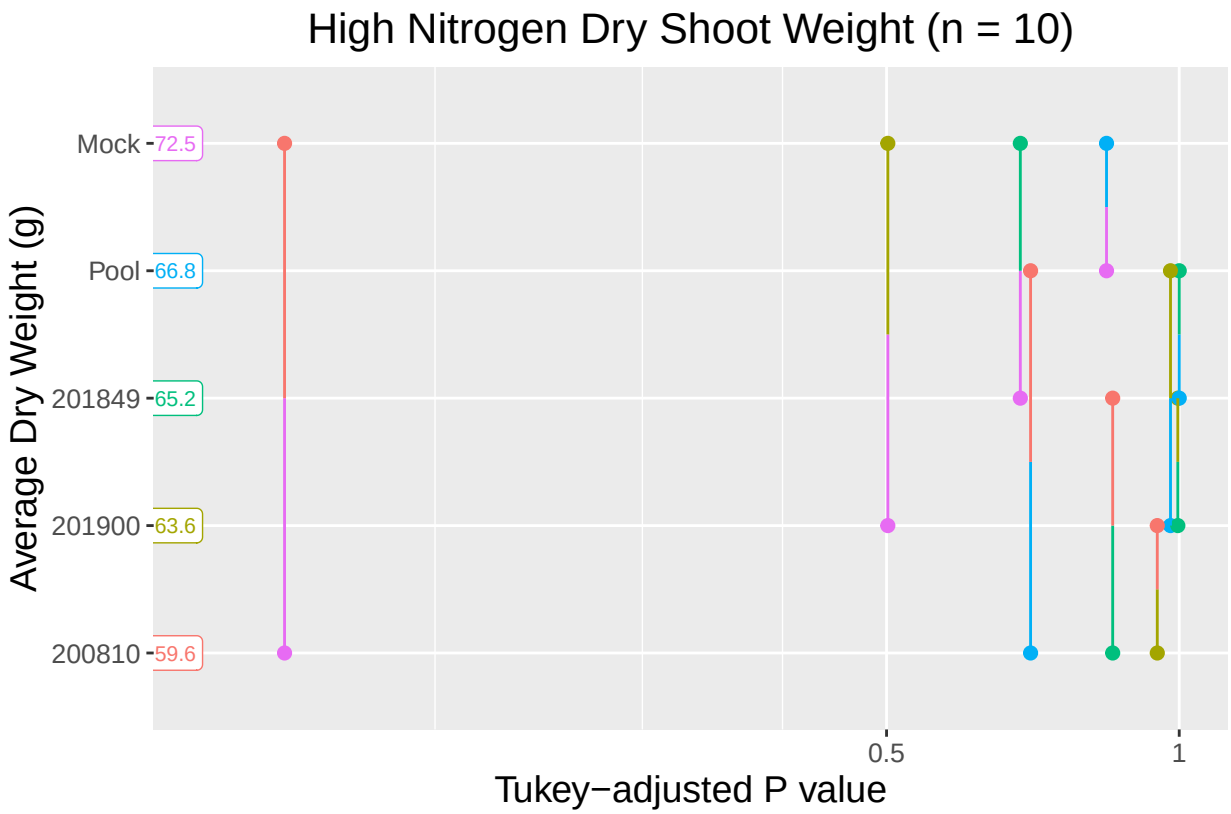
- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849    -5.60 5.51 45 -1.017  0.8462
##  200810 - 201900    -4.05 5.51 45 -0.735  0.9470
```

```
##  200810 - Mock     -12.90 5.51 45 -2.343  0.1506
##  200810 - Pool      -7.15 5.51 45 -1.298  0.6935
##  201849 - 201900     1.55 5.51 45  0.281  0.9986
##  201849 - Mock      -7.30 5.51 45 -1.326  0.6769
##  201849 - Pool      -1.55 5.51 45 -0.281  0.9986
##  201900 - Mock      -8.85 5.51 45 -1.607  0.5008
##  201900 - Pool      -3.10 5.51 45 -0.563  0.9797
##  Mock - Pool         5.75 5.51 45  1.044  0.8334
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

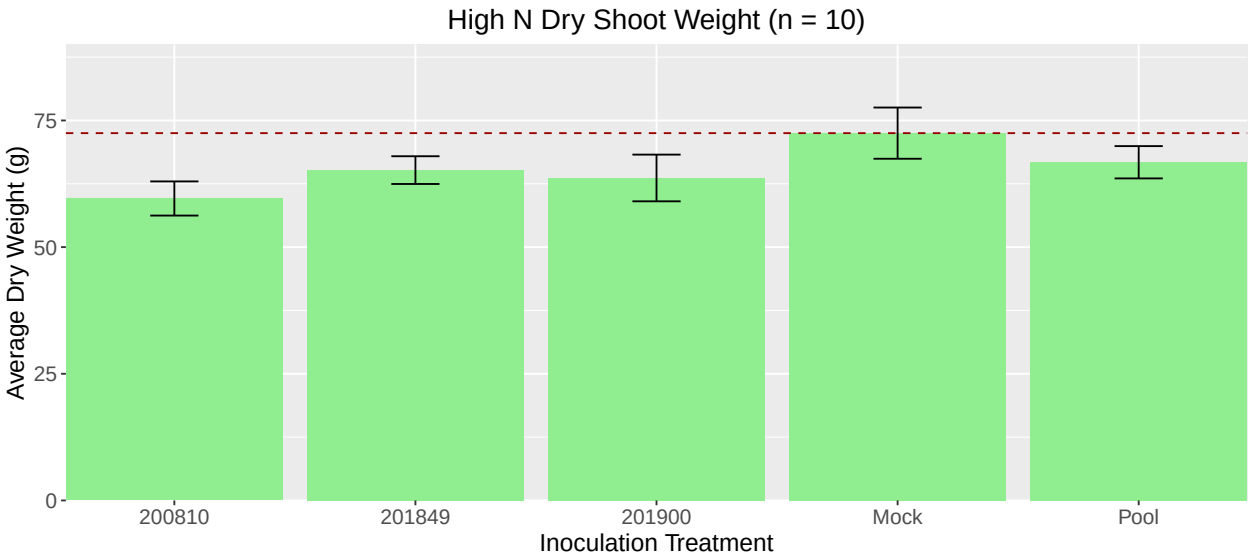




Mock-inoculated plants had the highest average dry shoot weight, but confidence levels in the difference estimates are low with the entire dataset.

Bar Plot

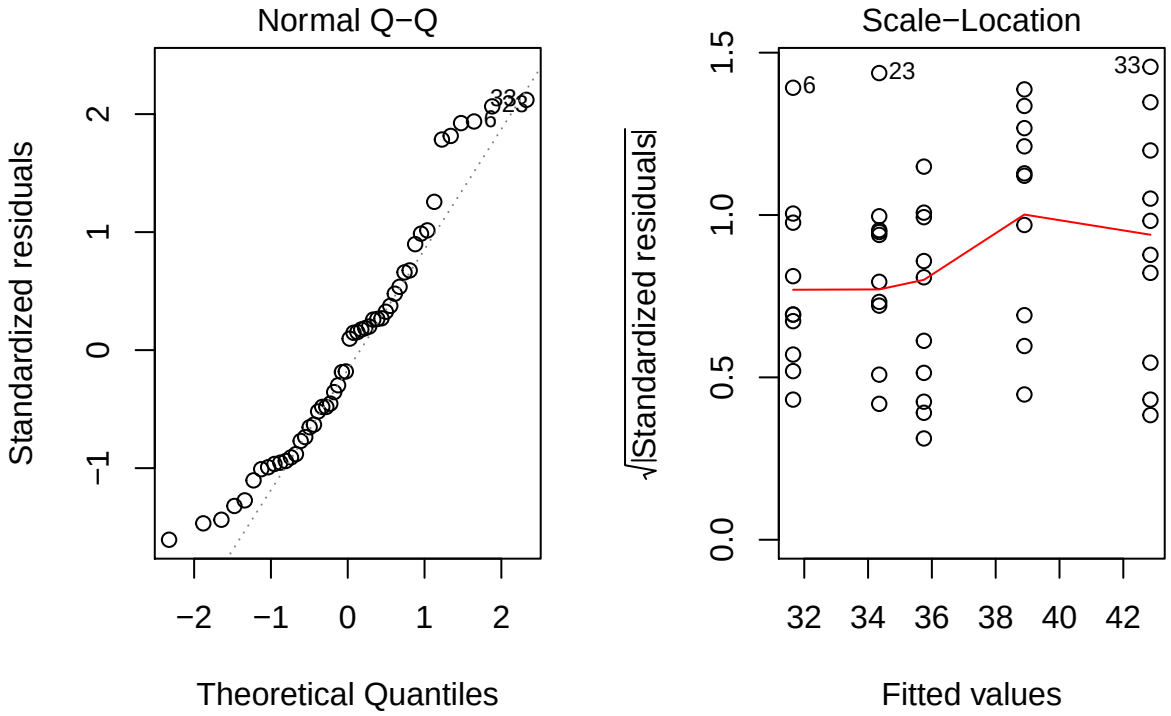
n=10



Mock-inoculated plants had the highest average dry shoot weight when analyzing the entire dataset.

Root Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: root_dry_wt_g
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4   745.9  186.48  0.5187 0.7224
## Residuals 45 16177.1  359.49
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

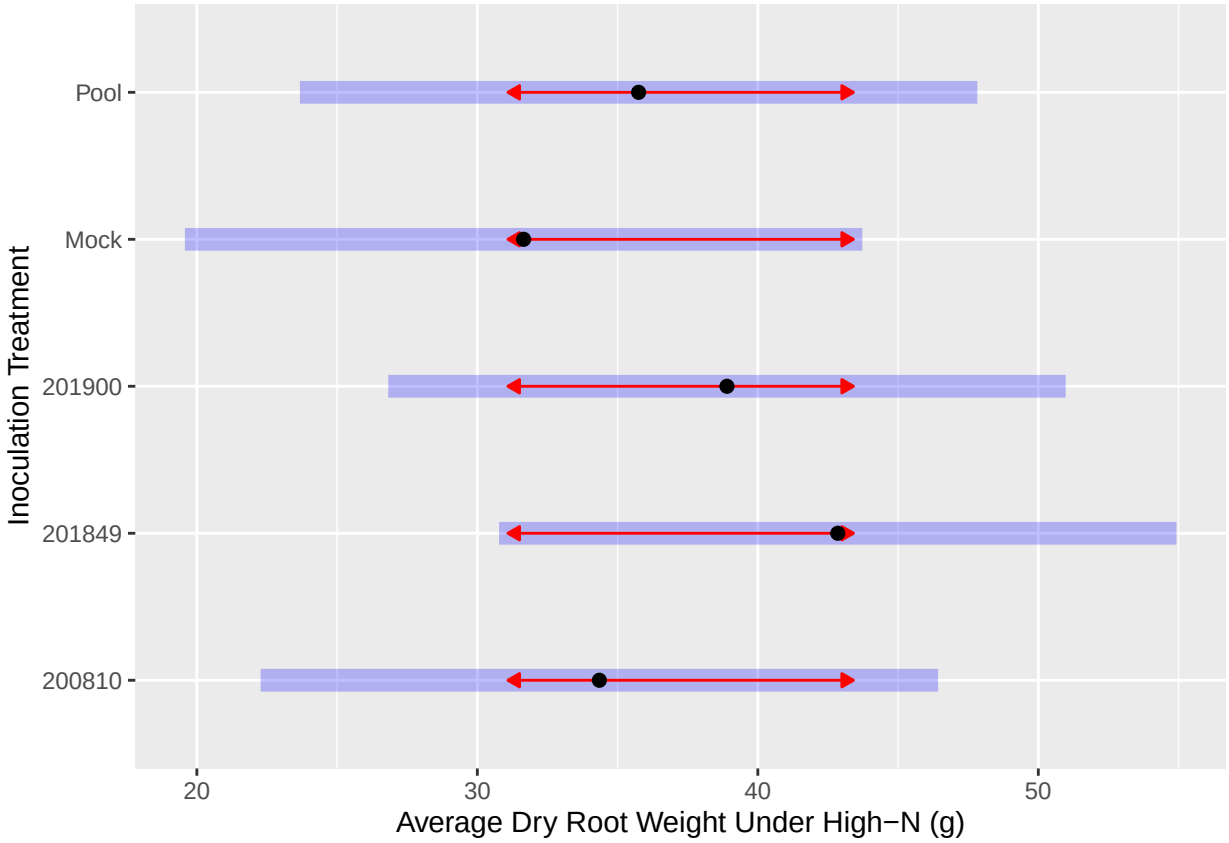
Tukey method of correction for multiple testing

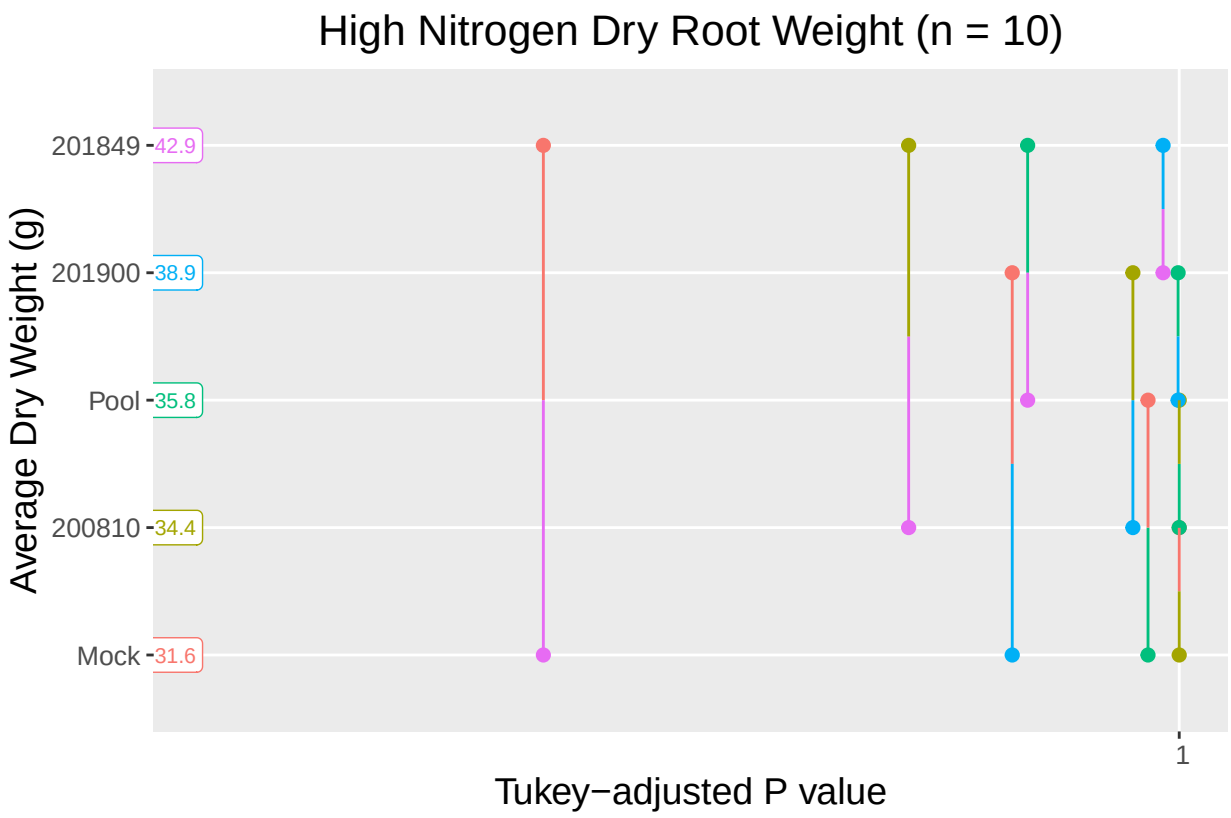
- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849    -8.50 8.48 45 -1.002  0.8529
##  200810 - 201900    -4.55 8.48 45 -0.537  0.9830
##  200810 - Mock       2.70 8.48 45  0.318  0.9977
##  200810 - Pool      -1.40 8.48 45 -0.165  0.9998
##  201849 - 201900     3.95 8.48 45  0.466  0.9900
##  201849 - Mock      11.20 8.48 45  1.321  0.6799
##  201849 - Pool       7.10 8.48 45  0.837  0.9175
##  201900 - Mock       7.25 8.48 45  0.855  0.9116
##  201900 - Pool       3.15 8.48 45  0.371  0.9958
##  Mock - Pool        -4.10 8.48 45 -0.484  0.9885
##
```

```
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

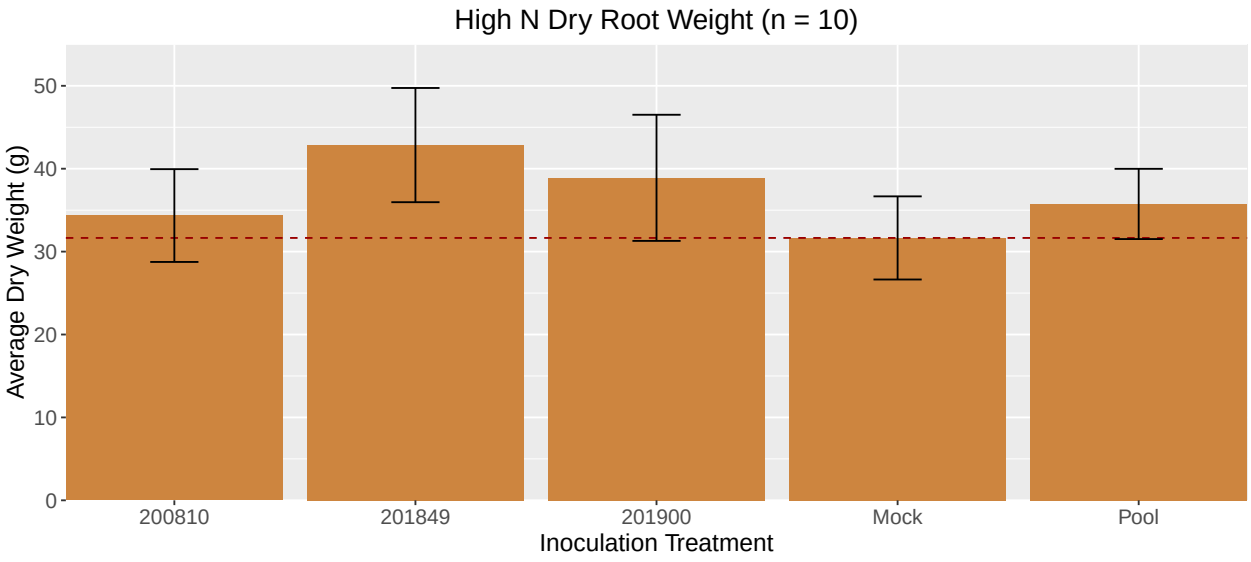




Statistical analysis of mean estimates for dry root weights of plants under high nitrogen fertilization indicate that confidence in observed mean differences among treatment groups is low. However, all inoculated groups have higher average dry root weight than the mock-inoculated group.

Bar Plot

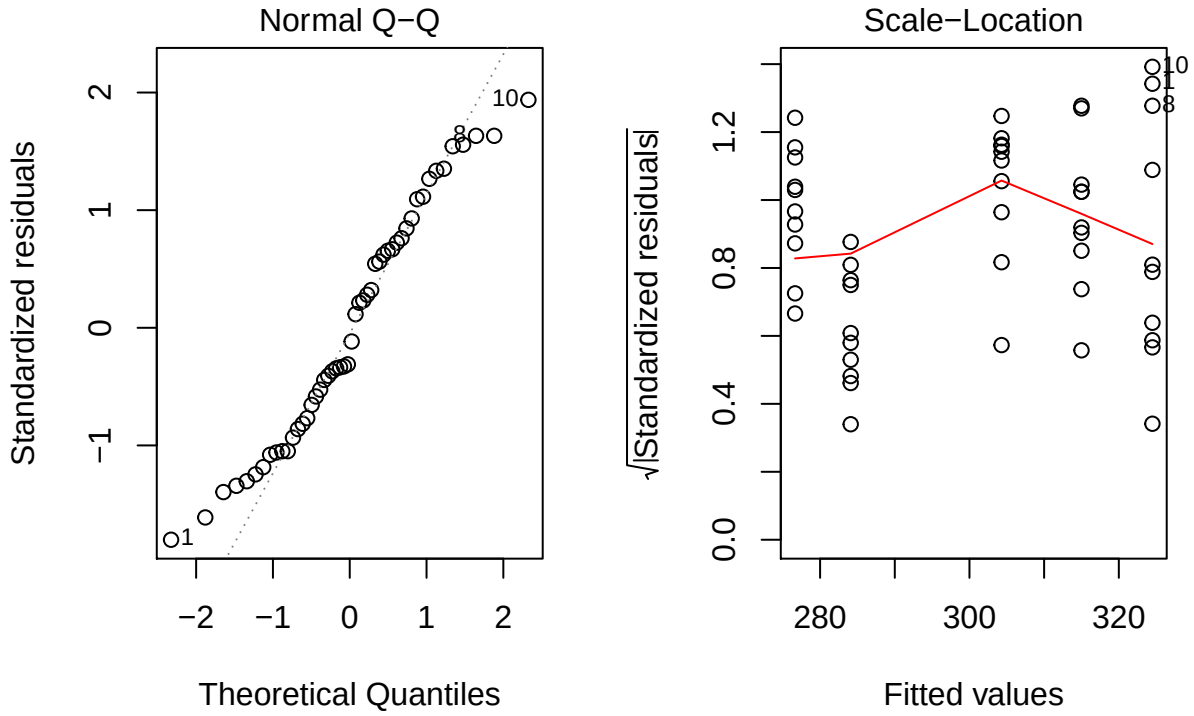
n=10



201849 and 201900-inoculated plants had higher mean dry root weights than the mock-inoculated plants, but the error bars are overlapping.

Tuber Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: tuber_wt_g
##           Df Sum Sq Mean Sq F value Pr(>F)
## inoc       4  16376  4094.1  0.3477 0.8443
## Residuals 45 529904 11775.6
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

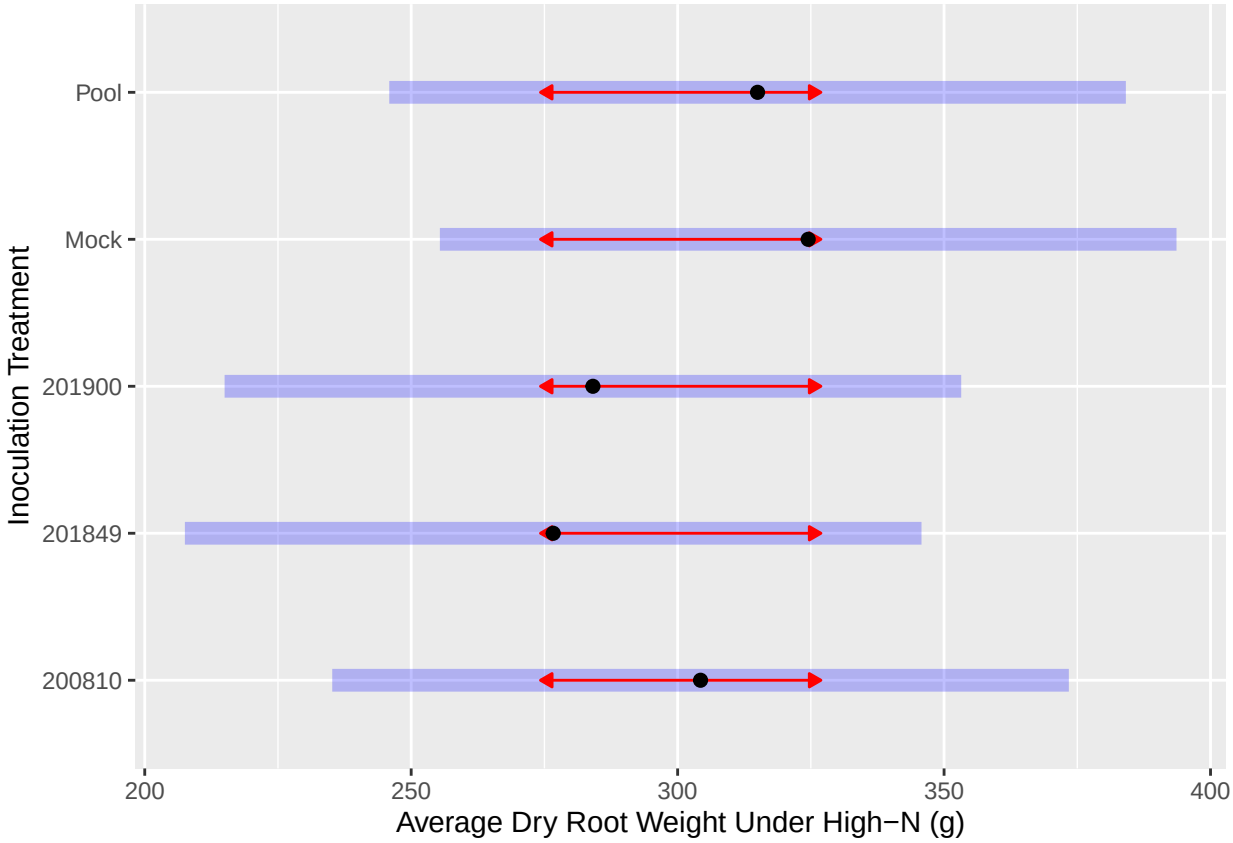
Tukey method of correction for multiple testing

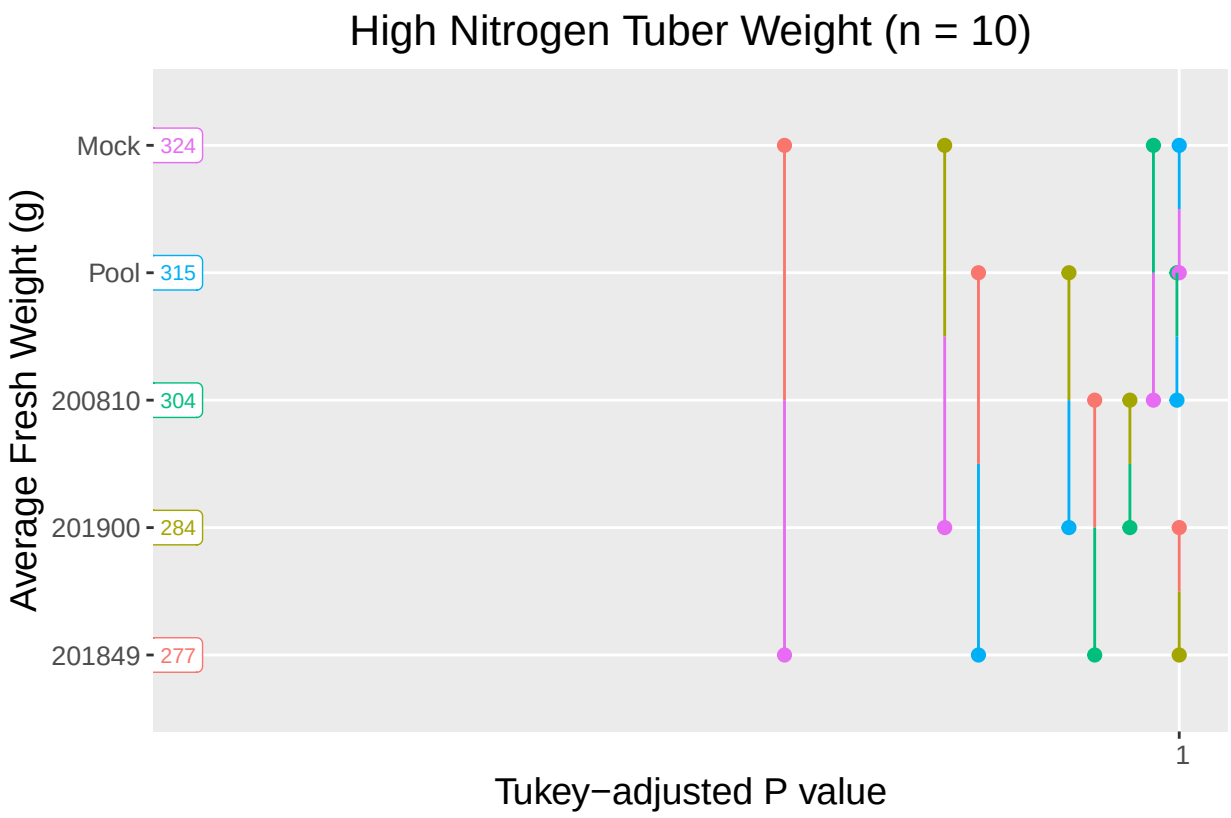
- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849    27.65 48.5 45  0.570  0.9788
##  200810 - 201900    20.20 48.5 45  0.416  0.9935
##  200810 - Mock     -20.20 48.5 45 -0.416  0.9935
##  200810 - Pool     -10.70 48.5 45 -0.220  0.9995
##  201849 - 201900    -7.45 48.5 45 -0.154  0.9999
##  201849 - Mock     -47.85 48.5 45 -0.986  0.8602
##  201849 - Pool     -38.35 48.5 45 -0.790  0.9321
##  201900 - Mock     -40.40 48.5 45 -0.832  0.9191
##  201900 - Pool     -30.90 48.5 45 -0.637  0.9682
##  Mock - Pool         9.50 48.5 45  0.196  0.9997
##
```

```
## P value adjustment: tukey method for comparing a family of 5 estimates
```

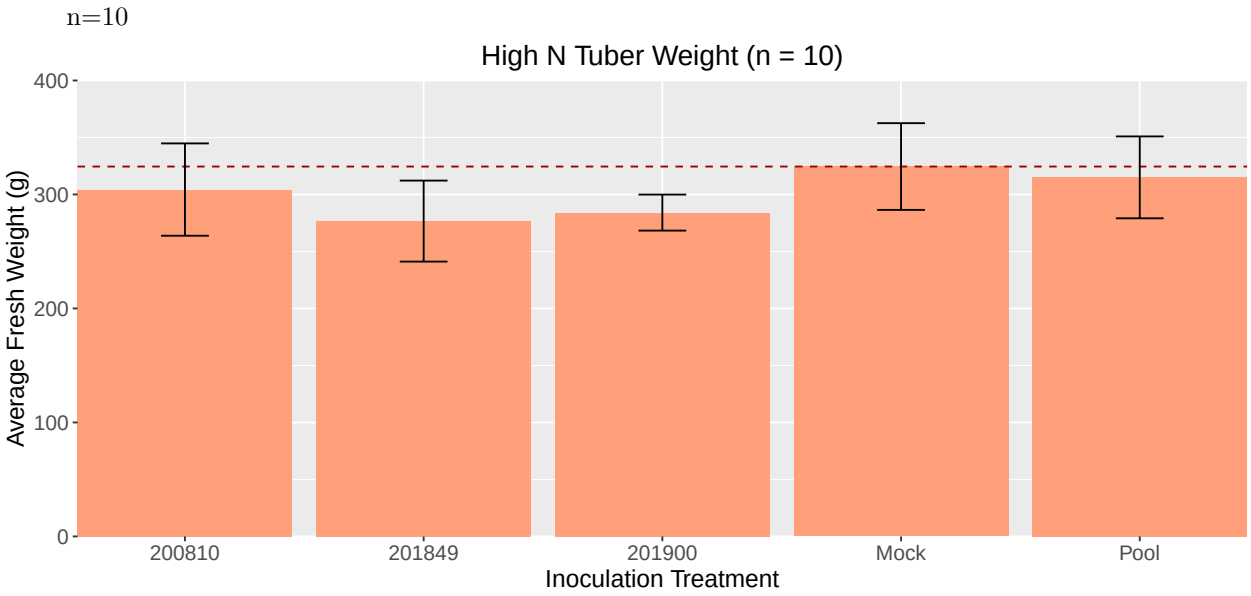
```
## pdf
##   2
```





When analyzing the full dataset, inoculation with microbes did not produce average tuber weights that were significantly higher than that of the mock-inoculated treatment group.

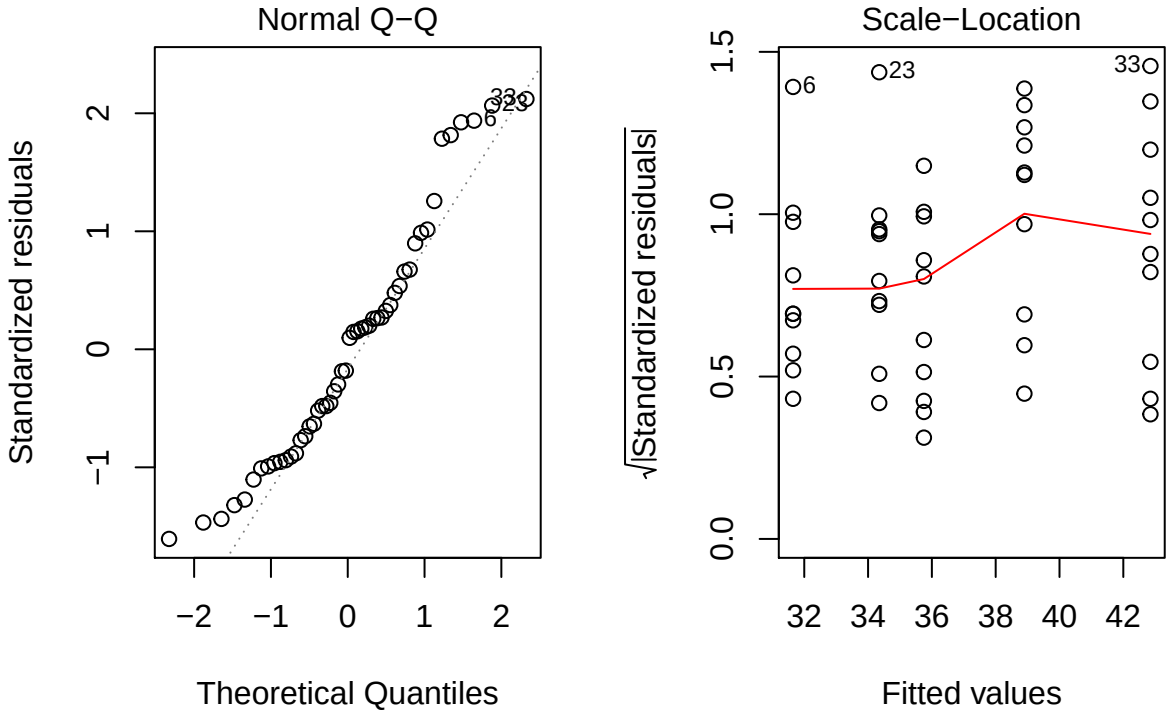
Bar Plot



Based on the full dataset, mock-inoculated potato plants produced the highest tuber weight.

Tuber Number

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: n_tuber
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4   22.52   5.630   0.145 0.9643
## Residuals 45 1747.50  38.833
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

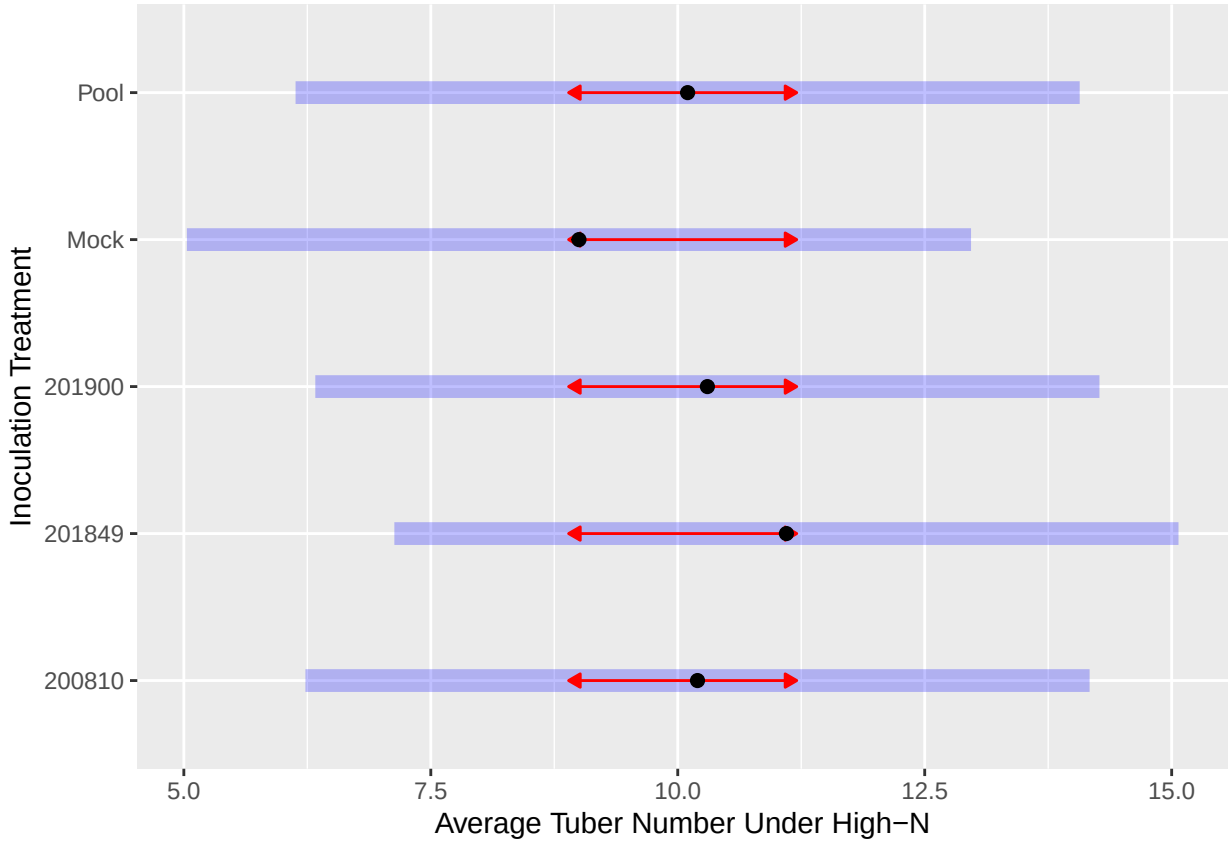
Tukey method of correction for multiple testing

- Alpha Level = 0.05

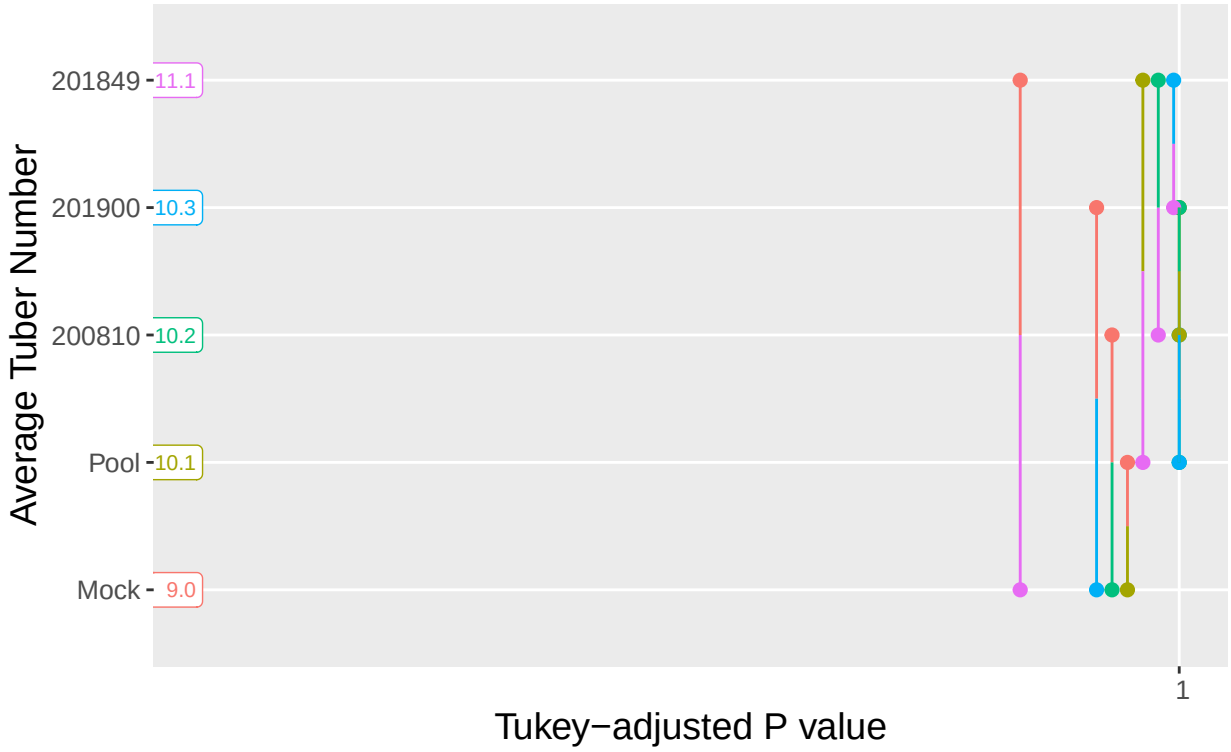
```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849     -0.9 2.79 45 -0.323  0.9976
##  200810 - 201900     -0.1 2.79 45 -0.036  1.0000
##  200810 - Mock        1.2 2.79 45  0.431  0.9926
##  200810 - Pool        0.1 2.79 45  0.036  1.0000
##  201849 - 201900      0.8 2.79 45  0.287  0.9985
##  201849 - Mock        2.1 2.79 45  0.754  0.9424
##  201849 - Pool        1.0 2.79 45  0.359  0.9963
##  201900 - Mock        1.3 2.79 45  0.466  0.9900
##  201900 - Pool        0.2 2.79 45  0.072  1.0000
##  Mock - Pool         -1.1 2.79 45 -0.395  0.9947
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
```

```
##   2
```



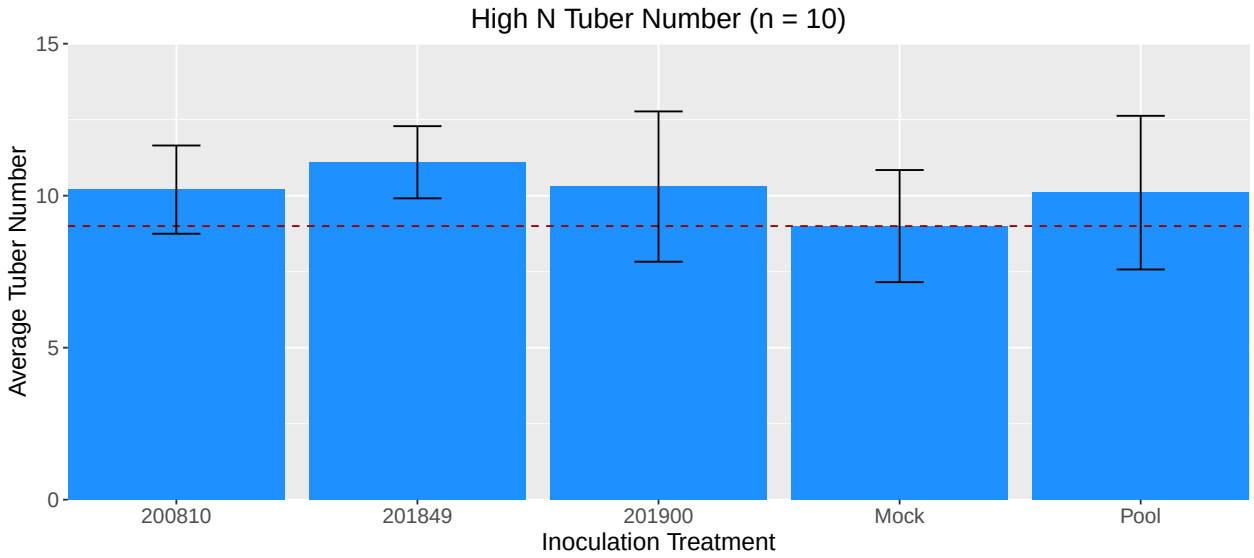
High Nitrogen Tuber Number (n = 10)



Average number of tubers was higher in all inoculated treatment groups compared to the mock-inoculated control group, but the confidence levels in the mean estimates for this response variable are low.

Bar Plot

n=10



Analysis of the complete dataset suggests that there were no observed meaningful differences in average tuber number among the treatment groups.

Low Nitrogen Treatment

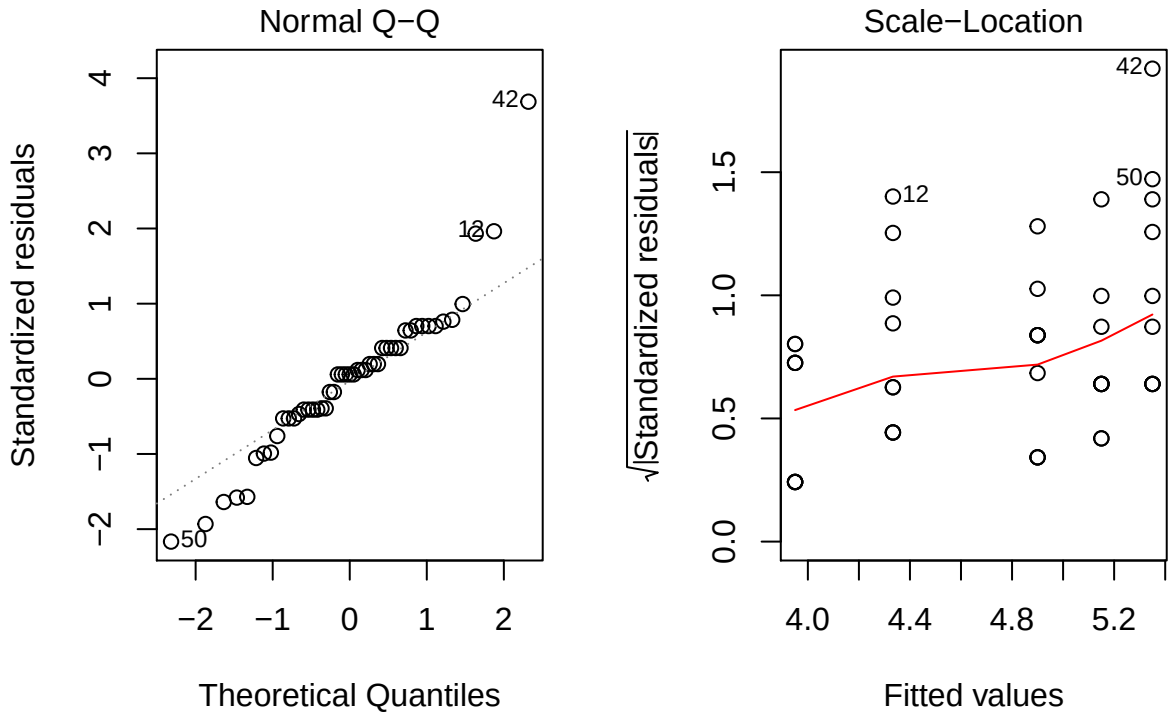
```
## Classes 'spec_tbl_df', 'tbl_df', 'tbl' and 'data.frame': 50 obs. of  12 variables:
##  $ sample          : chr  "A-N2-1.1" "A-N2-1.2" "A-N2-2.1" "A-N2-2.2" ...
##  $ nitrogen        : chr  "N2" "N2" "N2" "N2" ...
##  $ inoc            : chr  "Mock" "Mock" "Mock" "Mock" ...
##  $ group           : Factor w/ 5 levels "A","B","C","D",..: 1 1 1 1 1 1 1 1 1 1 ...
##  $ plot            : Factor w/ 5 levels "R1","R2","R3",..: 1 1 2 2 3 3 4 4 5 5 ...
##  $ rep             : Factor w/ 2 levels "1","2": 1 2 1 2 1 2 1 2 1 2 ...
##  $ n_tuber         : num  2 1 2 5 5 3 6 2 3 6 ...
##  $ tuber_wt_g      : num  30 53.5 64 3 44 63 49 69.5 39 61.5 ...
##  $ shoot_dry_wt_bag: num  47 47.5 47 46.5 47 47 47 46.5 46.5 47.5 ...
##  $ shoot_dry_wt_g  : num  4 4.5 4 3.5 4 4 4 3.5 3.5 4.5 ...
##  $ root_dry_wt_g   : num  15.5 12 11 15.5 16.5 9 16 10.5 15 9.5 ...
##  $ Comments        : chr  NA NA NA "all green" ...
##  - attr(*, "problems")=Classes 'tbl_df', 'tbl' and 'data.frame': 1 obs. of  5 variables:
##   ..$ row     : int 14
##   ..$ col     : chr "shoot_dry_wt_g"
##   ..$ expected: chr "a number"
##   ..$ actual  : chr "#VALUE!"
##   ..$ file    : chr "'./data_csv/2019_CPRAB_low_N_data.csv'"
##  - attr(*, "spec")=
##   .. cols(
##   ..   sample = col_character(),
##   ..   nitrogen = col_character(),
```

```
##   ..   inoc = col_character(),
##   ..   group = col_factor(levels = NULL, ordered = FALSE, include_na = FALSE),
##   ..   plot = col_factor(levels = NULL, ordered = FALSE, include_na = FALSE),
##   ..   rep = col_factor(levels = NULL, ordered = FALSE, include_na = FALSE),
##   ..   n_tuber = col_number(),
##   ..   tuber_wt_g = col_number(),
##   ..   shoot_dry_wt_bag = col_number(),
##   ..   shoot_dry_wt_g = col_number(),
##   ..   root_dry_wt_g = col_number(),
##   ..   Comments = col_character()
##   .. )
```

```
##     sample            nitrogen            inoc           group  plot
##  Length:50          Length:50          Length:50          A:10   R1:10
##  Class :character   Class :character   Class :character   B:10   R2:10
##  Mode  :character   Mode  :character   Mode  :character   C:10   R3:10
##                                                           D:10   R4:10
##                                                           E:10   R5:10
##
##
##  rep       n_tuber         tuber_wt_g    shoot_dry_wt_bag shoot_dry_wt_g
##  1:25   Min.   : 1.000   Min.   : 3.00   Min.   :46.00    Min.   :3.000
##  2:25   1st Qu.: 2.000   1st Qu.:39.00   1st Qu.:47.00    1st Qu.:4.000
##         Median : 4.000   Median :52.50   Median :47.50    Median :4.500
##         Mean   : 4.245   Mean   :51.67   Mean   :47.74    Mean   :4.745
##         3rd Qu.: 6.000   3rd Qu.:63.00   3rd Qu.:48.50    3rd Qu.:5.500
##         Max.   :11.000   Max.   :89.00   Max.   :51.50    Max.   :8.500
##         NA's   :1        NA's   :1       NA's   :1        NA's   :1
##  root_dry_wt_g     Comments
##  Min.   : 7.50   Length:50
##  1st Qu.:13.00   Class :character
##  Median :16.00   Mode  :character
##  Mean   :17.24
##  3rd Qu.:18.50
##  Max.   :36.50
##  NA's   :1
```

Shoot Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: shoot_dry_wt_g
##           Df Sum Sq Mean Sq F value   Pr(>F)
## inoc       4 13.386  3.3466  4.1275 0.006324 **
## Residuals 44 35.675  0.8108
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimated Marginal Means Analysis

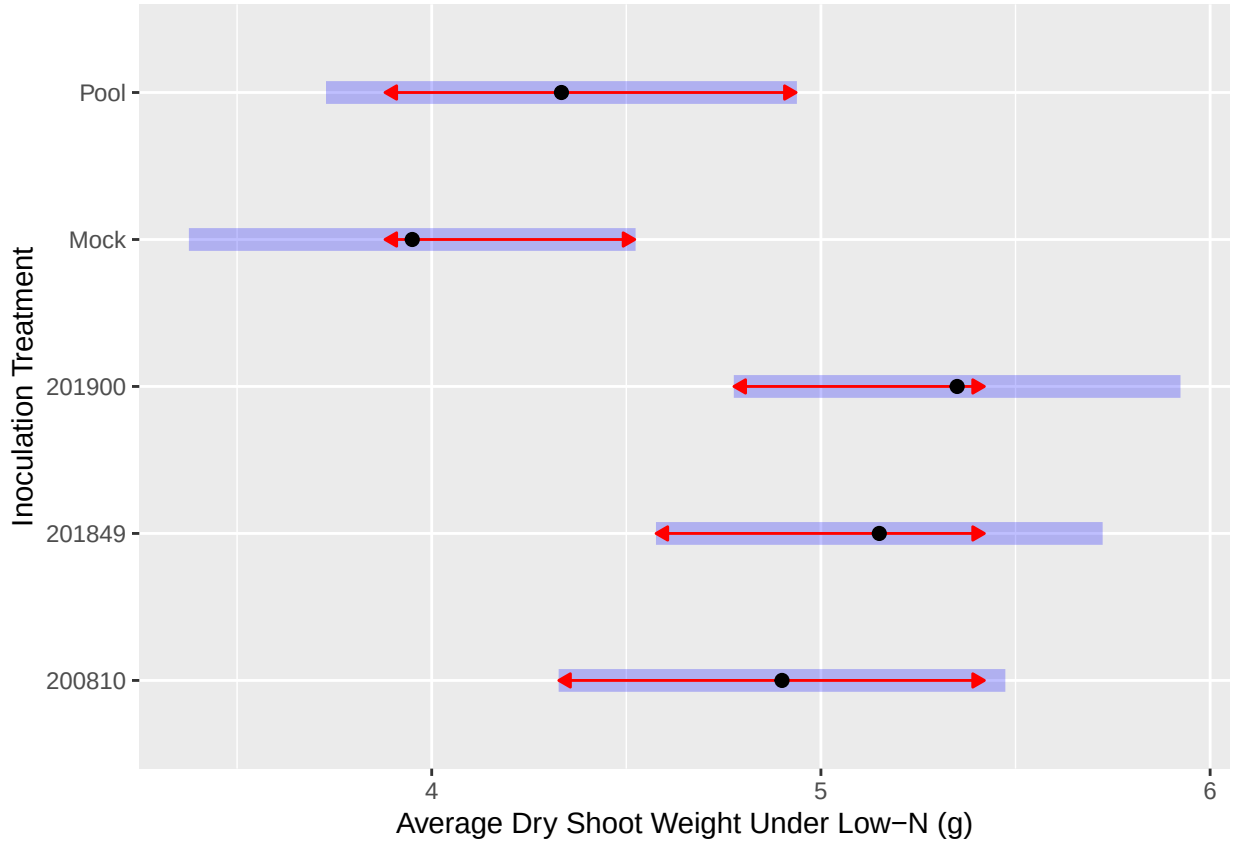
All Pair-wise Comparisons

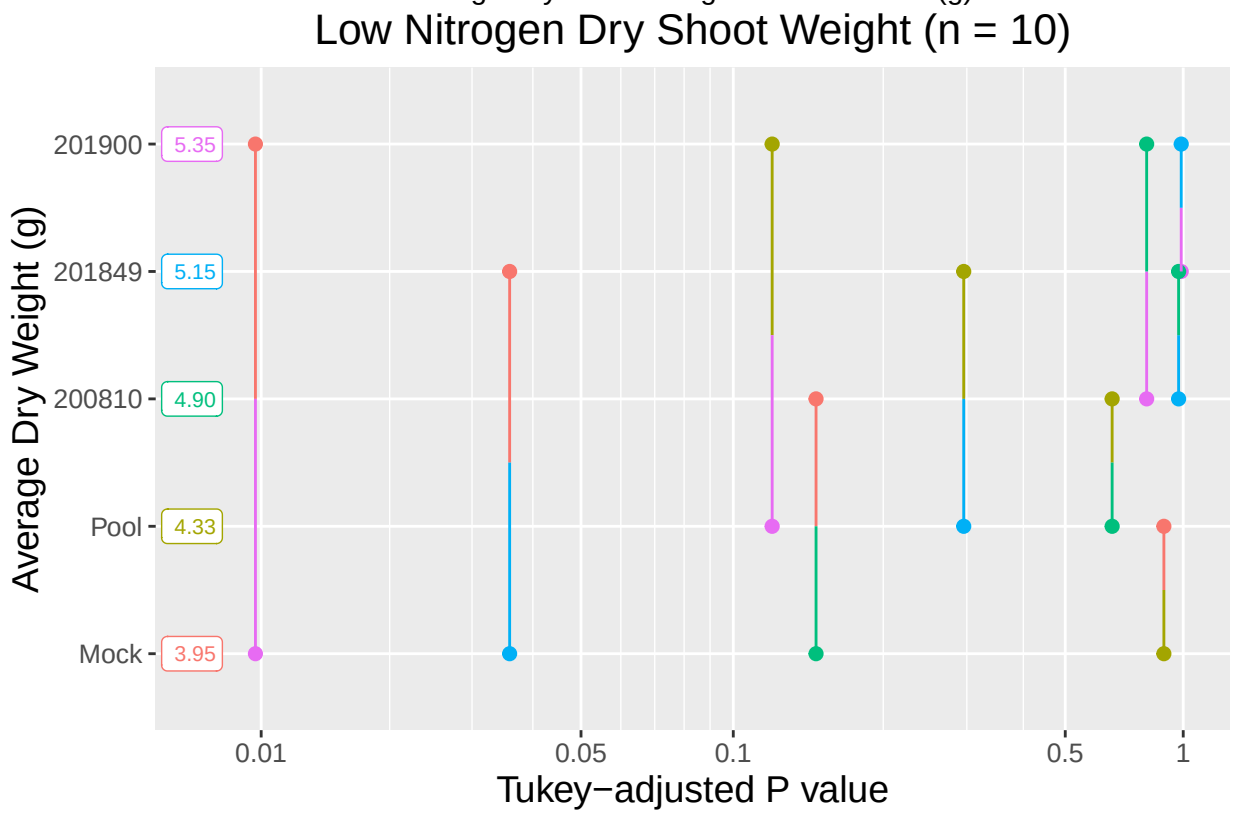
Tukey method of correction for multiple testing

- Alpha Level = 0.05

```
##  contrast        estimate    SE df t.ratio p.value
##  200810 - 201849   -0.250 0.403 44 -0.621  0.9710
##  200810 - 201900   -0.450 0.403 44 -1.117  0.7965
##  200810 - Mock      0.950 0.403 44  2.359  0.1461
##  200810 - Pool      0.567 0.414 44  1.370  0.6498
##  201849 - 201900   -0.200 0.403 44 -0.497  0.9873
##  201849 - Mock      1.200 0.403 44  2.980  0.0359
##  201849 - Pool      0.817 0.414 44  1.974  0.2954
##  201900 - Mock      1.400 0.403 44  3.477  0.0097
##  201900 - Pool      1.017 0.414 44  2.457  0.1194
##  Mock - Pool       -0.383 0.414 44 -0.927  0.8851
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

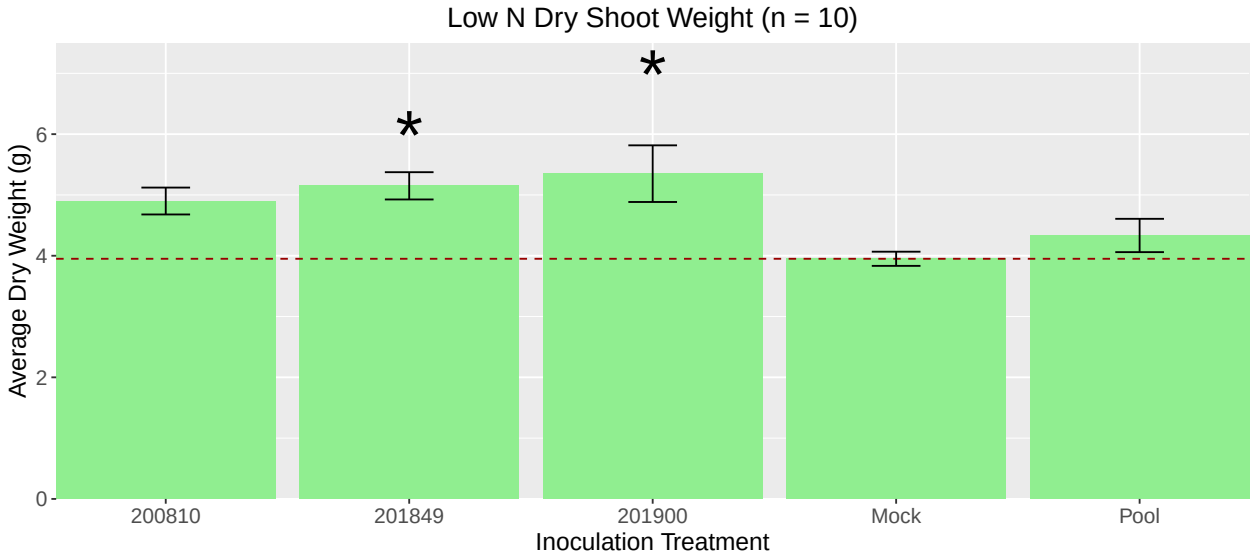

Pool
Mock
201900
201849
200810
Inoculation Treatment
4
5
6
Average Dry Shoot Weight Under Low−N (g)


Low Nitrogen Dry Shoot Weight (n = 10)
201900
5.35
201849
5.15
200810
4.90
Pool
4.33
Mock
3.95
Average Dry Weight (g)
0.01
0.05
0.1
0.5
1
Tukey−adjusted P value

Based on the experimental data, the estimated mean dry shoot weight of potato plants subjected to low N treatment while receiving mono-isolate inoculations with bacterial isolates is higher than that of the mock inoculated control plants. Plants under low-N that received the inoculation pool of 3 microbes have a mean shoot weight higher than the mock, but the confidence in the mean estimate difference is much lower. The P-values for the mean estimate comparisons between the mock control plants and those receiving 201849 and 201900 inoculations are both very low, which boosts the confidence in the estimated difference in mean shoot weight.

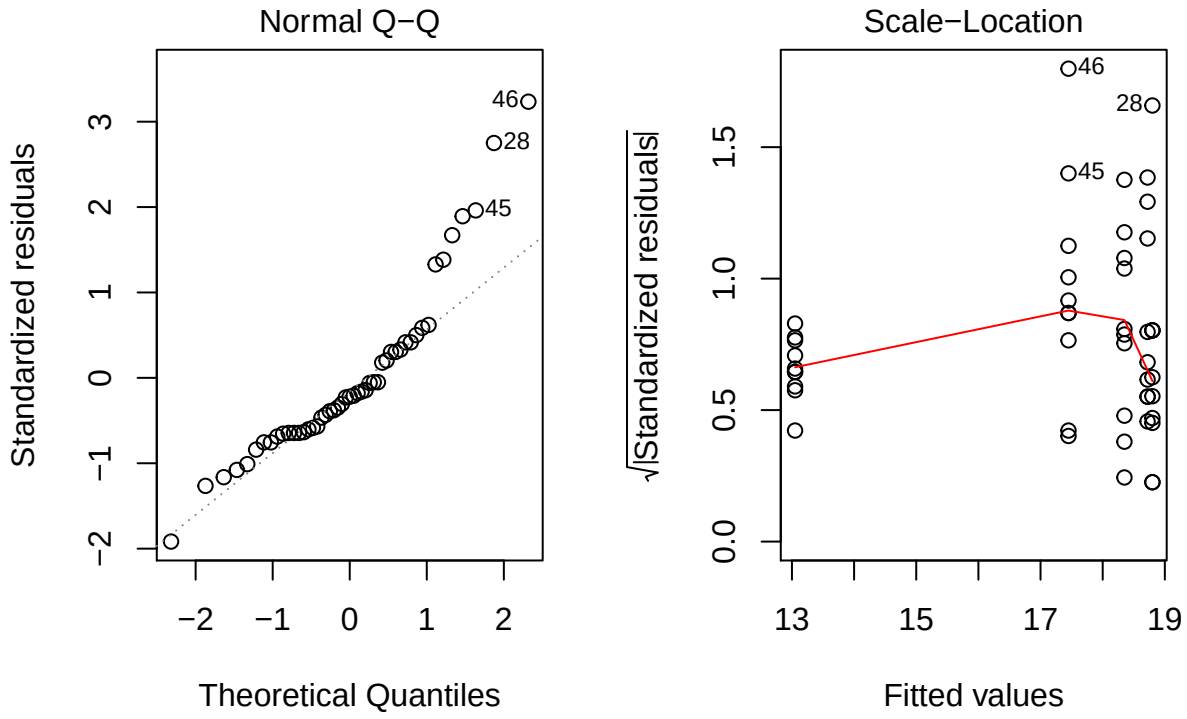
Bar Plot

n=10



Root Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: root_dry_wt_g
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4  232.43  58.108  1.5074 0.2165
## Residuals 44 1696.13  38.548
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

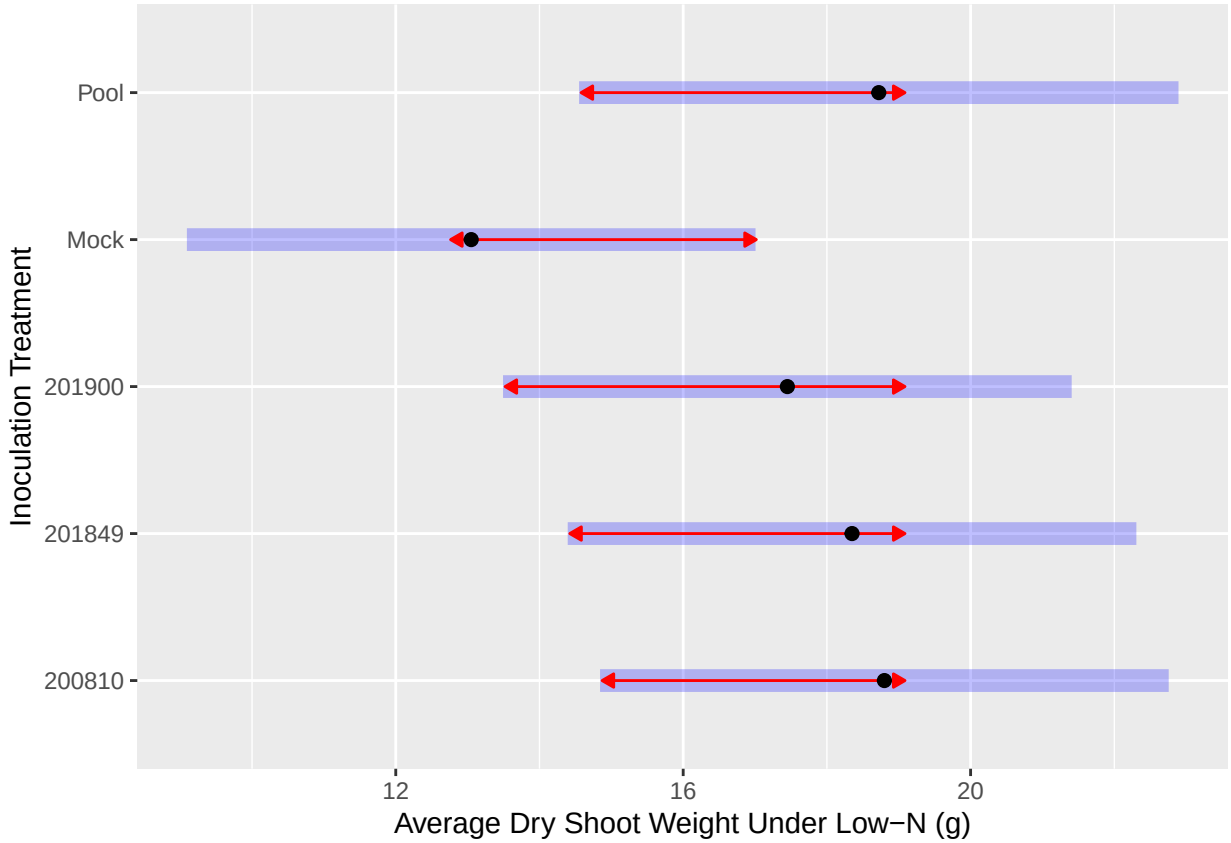
Tukey method of correction for multiple testing

- Alpha Level = 0.05

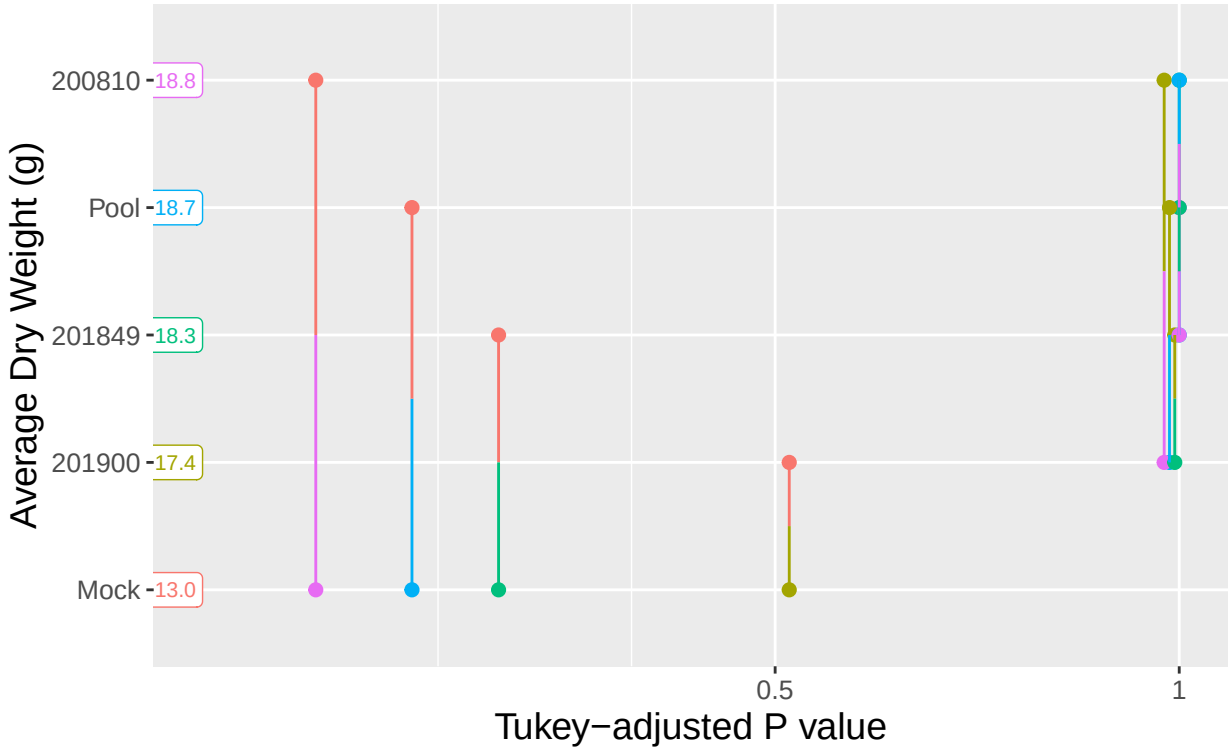
```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849   0.4500 2.78 44  0.162  0.9998
##  200810 - 201900   1.3500 2.78 44  0.486  0.9882
##  200810 - Mock     5.7500 2.78 44  2.071  0.2508
##  200810 - Pool     0.0778 2.85 44  0.027  1.0000
##  201849 - 201900   0.9000 2.78 44  0.324  0.9975
##  201849 - Mock     5.3000 2.78 44  1.909  0.3279
##  201849 - Pool    -0.3722 2.85 44 -0.130  0.9999
##  201900 - Mock     4.4000 2.78 44  1.585  0.5149
##  201900 - Pool    -1.2722 2.85 44 -0.446  0.9915
##  Mock - Pool      -5.6722 2.85 44 -1.988  0.2885
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
```

2



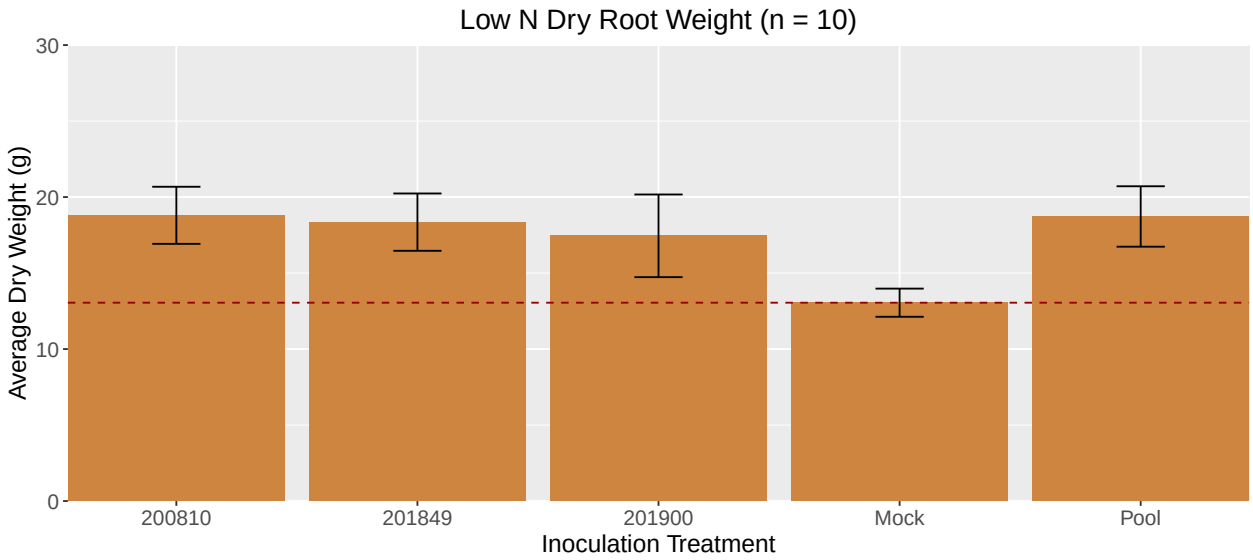
Low Nitrogen Dry Shoot Weight (n = 10)



Based on the experimental data, the estimated mean dry root weight of potato plants subjected to low N treatment with microbial inoculation are all higher than that of the mock inoculated control. However, the confidence levels for the mean estimate differences are low. The lower confidence intervals for the inoculated plant means overlap with the upper confidence interval of the mock inoculated plants. This decreases our confidence in the likelihood that the difference was due to random chance alone.

Bar Plot

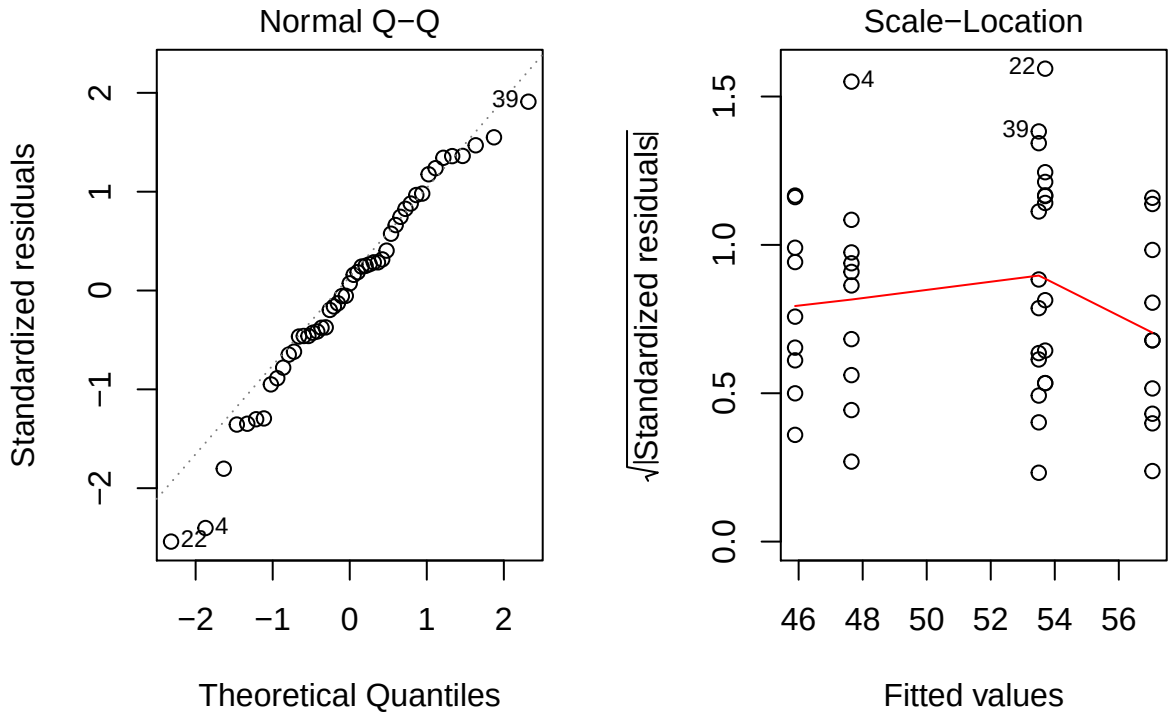
n=10



Average dry root weights were all higher for microbe inoculated plants compared to mock-inoculated controls. However, the confidence levels in the mean estimations are low due to high variance and adjusted p-values for the comparisons of treatment groups above.

Tuber Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: tuber_wt_g
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4   826.5  206.63  0.5387 0.7081
## Residuals 44 16877.7  383.58
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

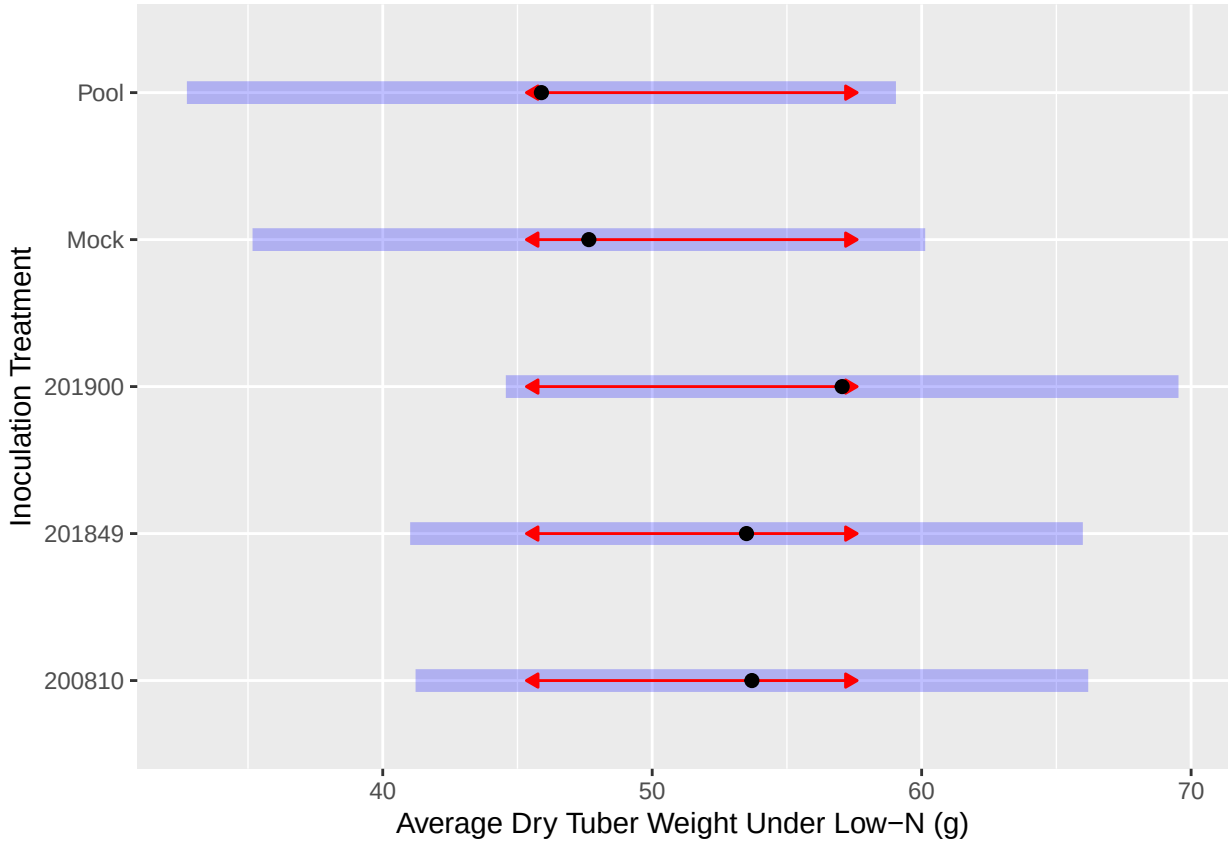
Tukey method of correction for multiple testing

- Alpha Level = 0.05

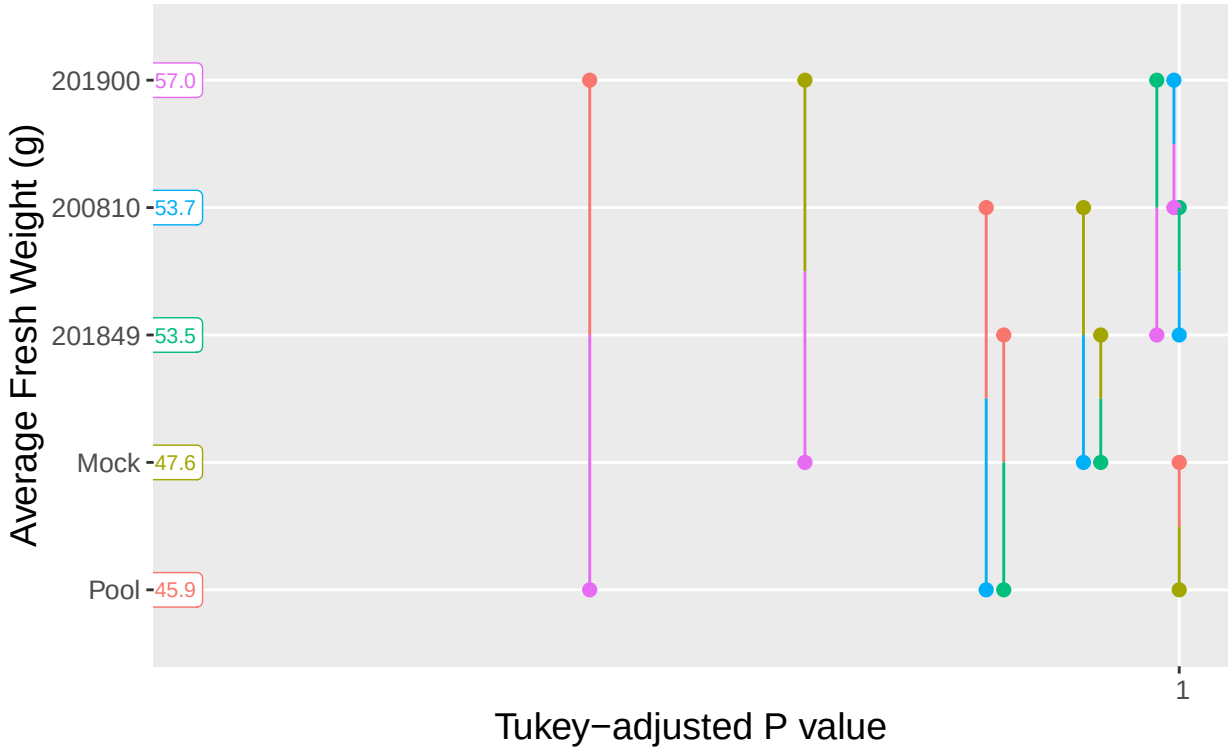
```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849     0.20 8.76 44  0.023  1.0000
##  200810 - 201900    -3.35 8.76 44 -0.382  0.9953
##  200810 - Mock       6.05 8.76 44  0.691  0.9575
##  200810 - Pool       7.81 9.00 44  0.868  0.9070
##  201849 - 201900    -3.55 8.76 44 -0.405  0.9941
##  201849 - Mock       5.85 8.76 44  0.668  0.9622
##  201849 - Pool       7.61 9.00 44  0.846  0.9147
##  201900 - Mock       9.40 8.76 44  1.073  0.8192
##  201900 - Pool      11.16 9.00 44  1.240  0.7281
##  Mock - Pool         1.76 9.00 44  0.196  0.9997
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
```

2



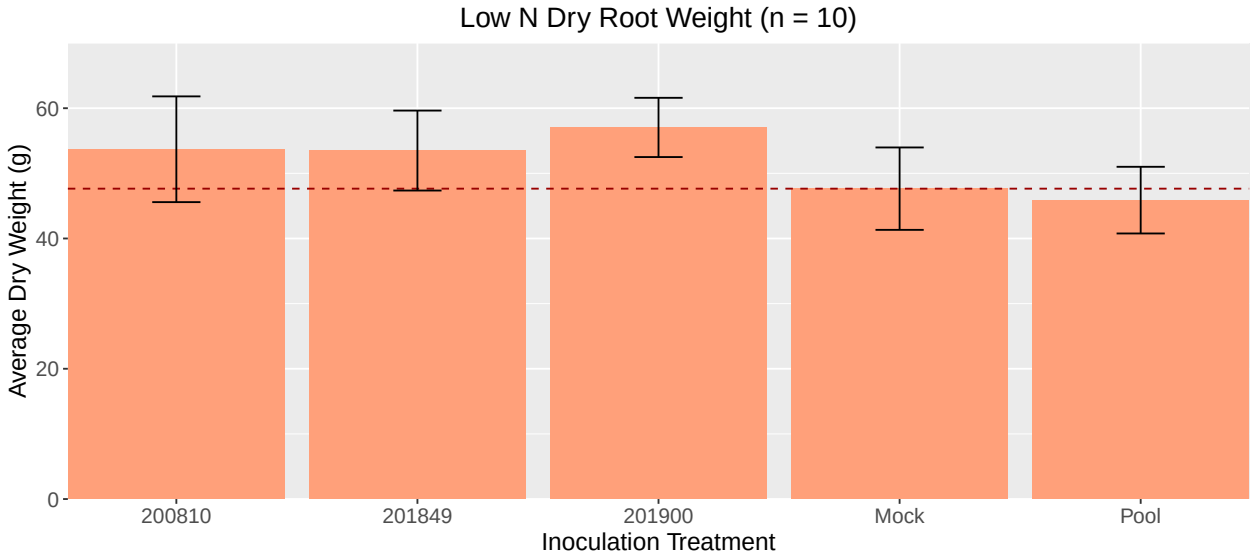
Low Nitrogen Tuber Weight (n = 10)



Based on the experimental data, the estimated mean dry tuber weight of potato plants subjected to low N treatment with microbial inoculation are all higher than that of the mock inoculated control. However, the confidence levels for the mean estimate differences are low. The lower confidence intervals for the inoculated plant means overlap with the upper confidence interval of the mock inoculated plants. This decreases our confidence in the likelihood that the difference was due to random chance alone.

Bar Plot

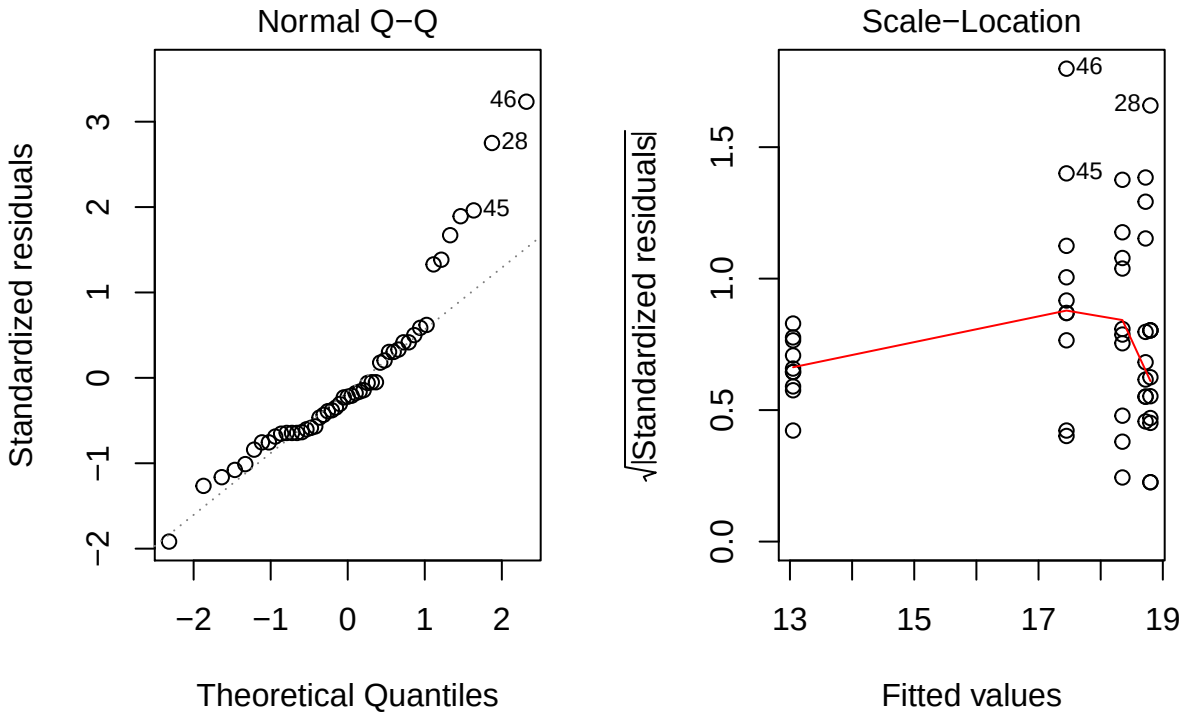
n=10



Mono-inoculated plants had higher average tuber weights than mock-inoculated and pool-inoculated groups, but confidence in the estimated differences of mean tuber weight is low when analyzing the full dataset.

Tuber Number

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: n_tuber
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4  41.006 10.2514  1.7212 0.1624
## Residuals 44 262.056  5.9558
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

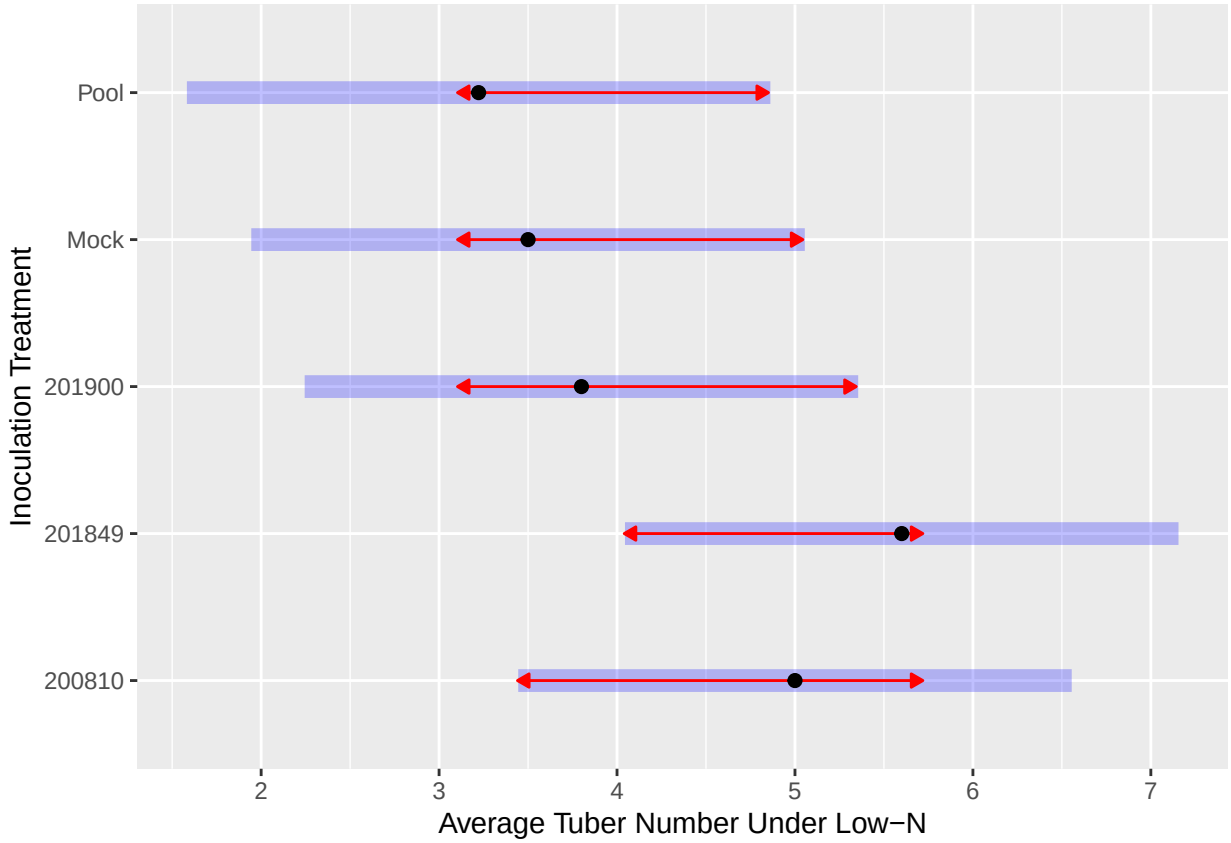
Tukey method of correction for multiple testing

- Alpha Level = 0.05

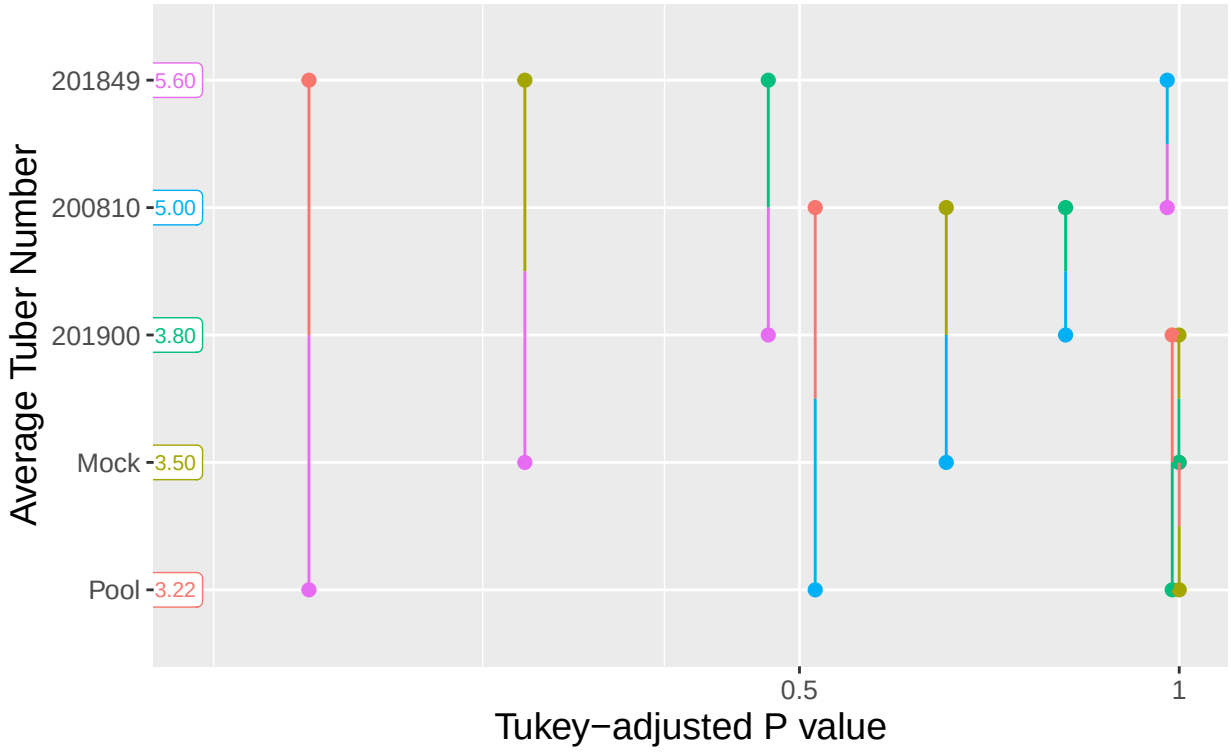
```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849   -0.600 1.09 44 -0.550  0.9814
##  200810 - 201900    1.200 1.09 44  1.100  0.8058
##  200810 - Mock      1.500 1.09 44  1.374  0.6469
##  200810 - Pool      1.778 1.12 44  1.585  0.5144
##  201849 - 201900    1.800 1.09 44  1.649  0.4751
##  201849 - Mock      2.100 1.09 44  1.924  0.3201
##  201849 - Pool      2.378 1.12 44  2.121  0.2298
##  201900 - Mock      0.300 1.09 44  0.275  0.9987
##  201900 - Pool      0.578 1.12 44  0.515  0.9854
##  Mock - Pool        0.278 1.12 44  0.248  0.9991
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
```

```
##   2
```



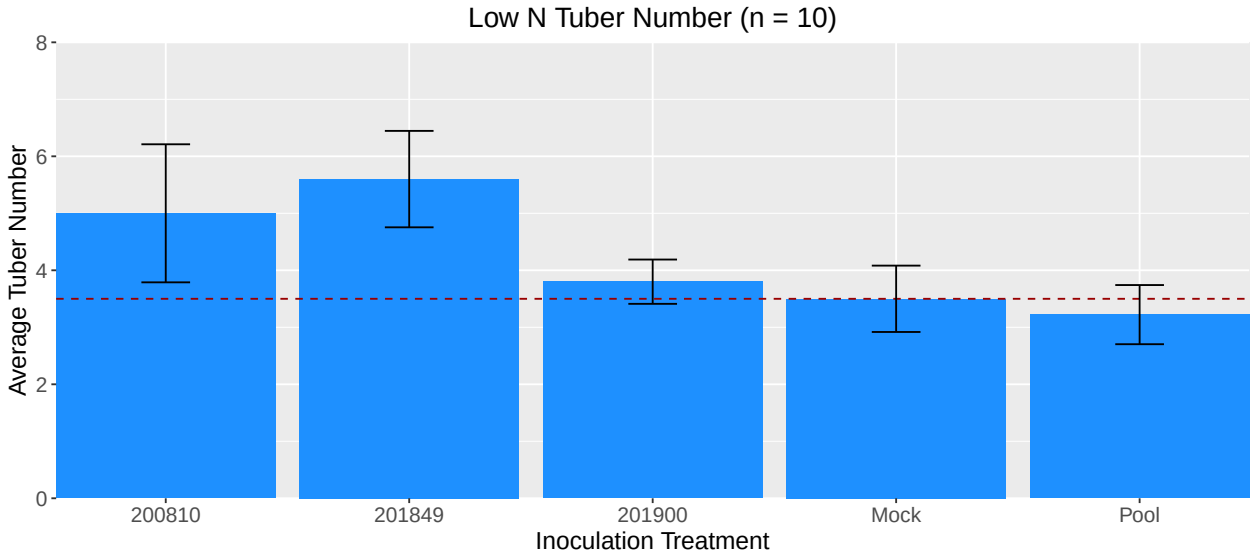
Low Nitrogen Tuber Number (n = 10)



Based on the full dataset, mono-inoculated plants under low nitrogen had higher average tuber numbers than mock-inoculated, while the pool-inoculated group had the lowest under the same fertilization conditions. Confidence in the estimations of the means is low based on analyzing the full dataset.

Bar Plot

n=10



While mono-inoculated groups all had higher average number of tubers per plant, the confidence in the comparison of mean-estimations between mono-inoculated groups and the mock-inoculated groups is relatively low based on the P-value for the comparisons.

Reduced Dataset

Remove Blocks 4 and 5

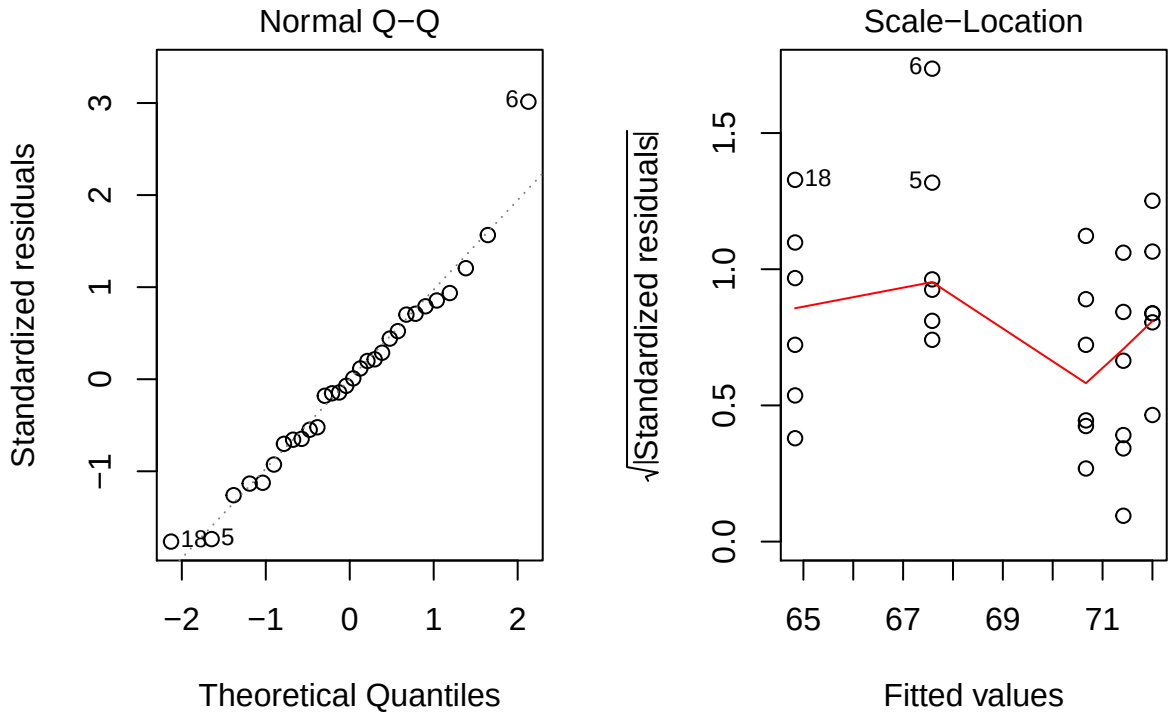
Blocks Four and Five experienced much colder climate conditions than Blocks One, Two and Three due to the temperature gradient in the greenhouse. Removing these two blocks reduces the number of plants for each treatment group from 10 to 6. The data will be re-analyzed after having subtracted these two randomized complete blocks.

High Nitrogen

n = 6 for each treatment group

Shoot Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: shoot_dry_wt_g
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4  219.22  54.804  0.5326  0.713
## Residuals 25 2572.58 102.903
```

Estimated Marginal Means Analysis

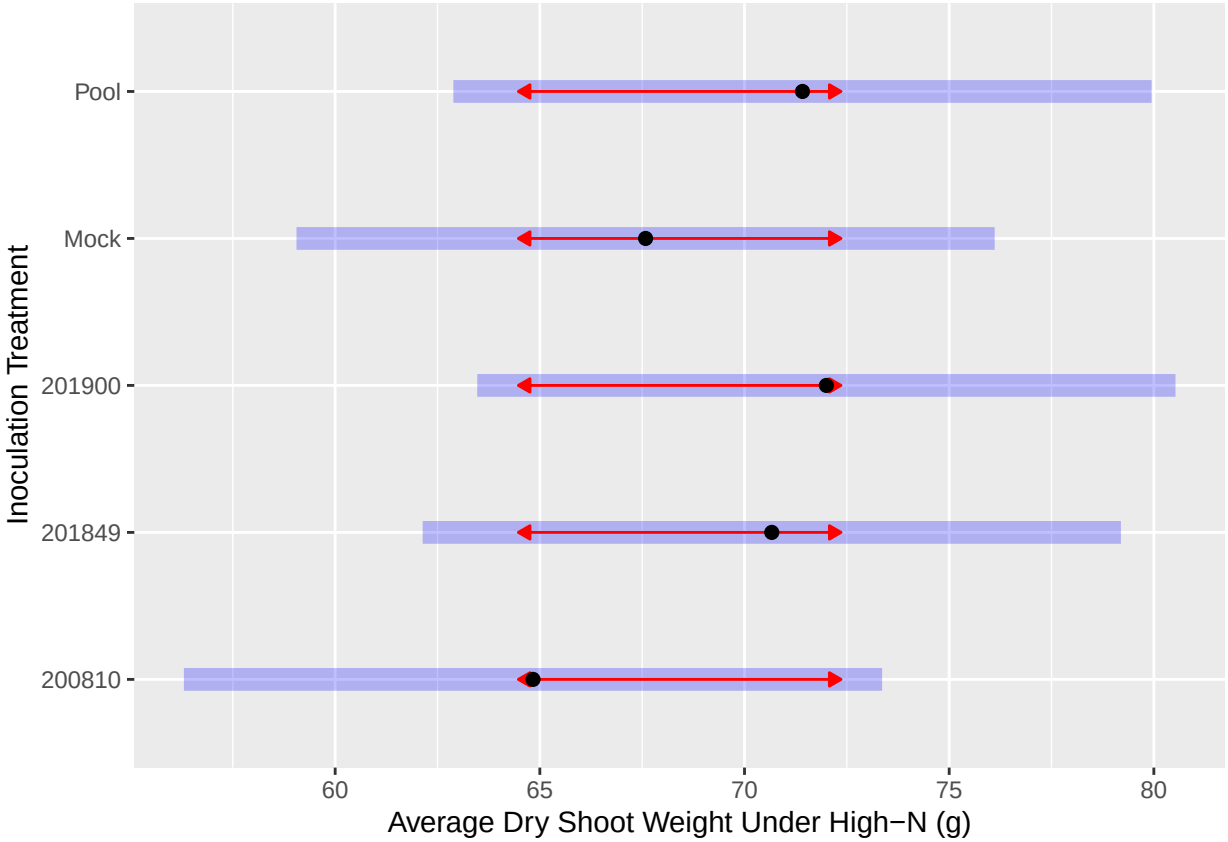
All Pair-wise Comparisons

Tukey method of correction for multiple testing

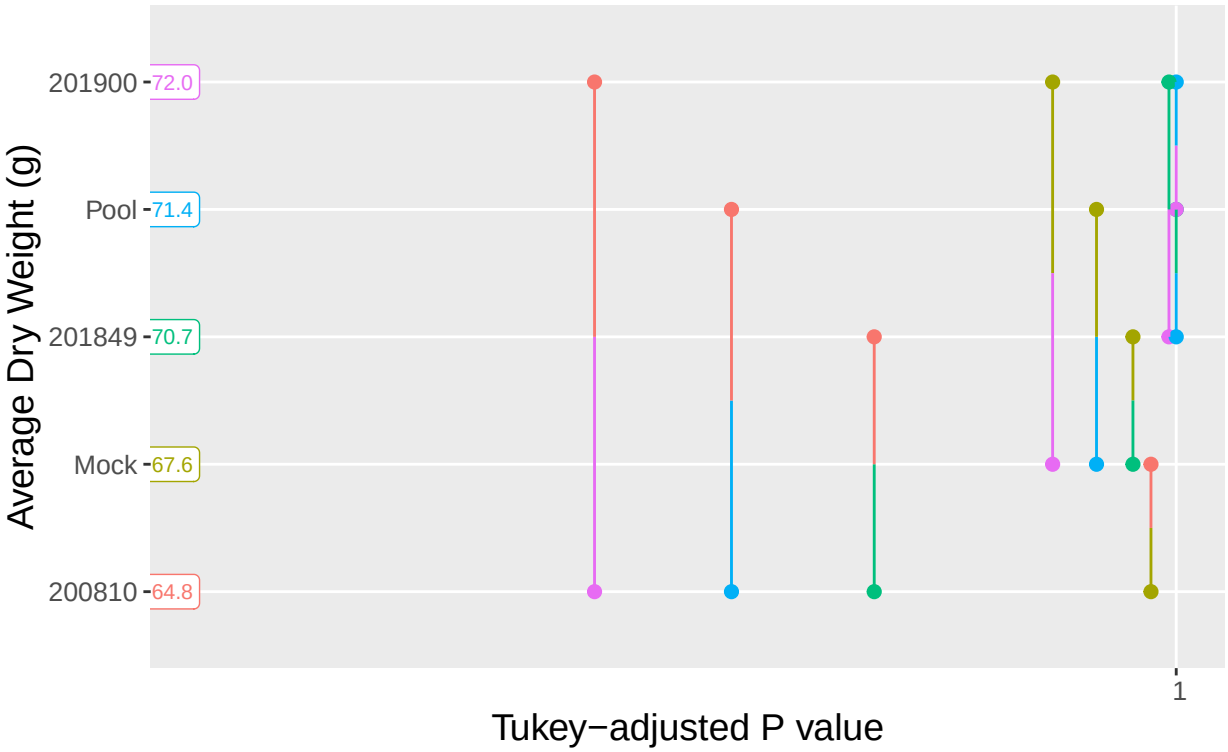
- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849   -5.833 5.86 25 -0.996  0.8546
##  200810 - 201900   -7.167 5.86 25 -1.224  0.7380
##  200810 - Mock     -2.750 5.86 25 -0.470  0.9894
##  200810 - Pool     -6.583 5.86 25 -1.124  0.7924
##  201849 - 201900   -1.333 5.86 25 -0.228  0.9994
##  201849 - Mock      3.083 5.86 25  0.526  0.9838
##  201849 - Pool     -0.750 5.86 25 -0.128  0.9999
##  201900 - Mock      4.417 5.86 25  0.754  0.9412
##  201900 - Pool      0.583 5.86 25  0.100  1.0000
##  Mock - Pool       -3.833 5.86 25 -0.655  0.9642
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```



High Nitrogen Dry Shoot Weight (n = 6)

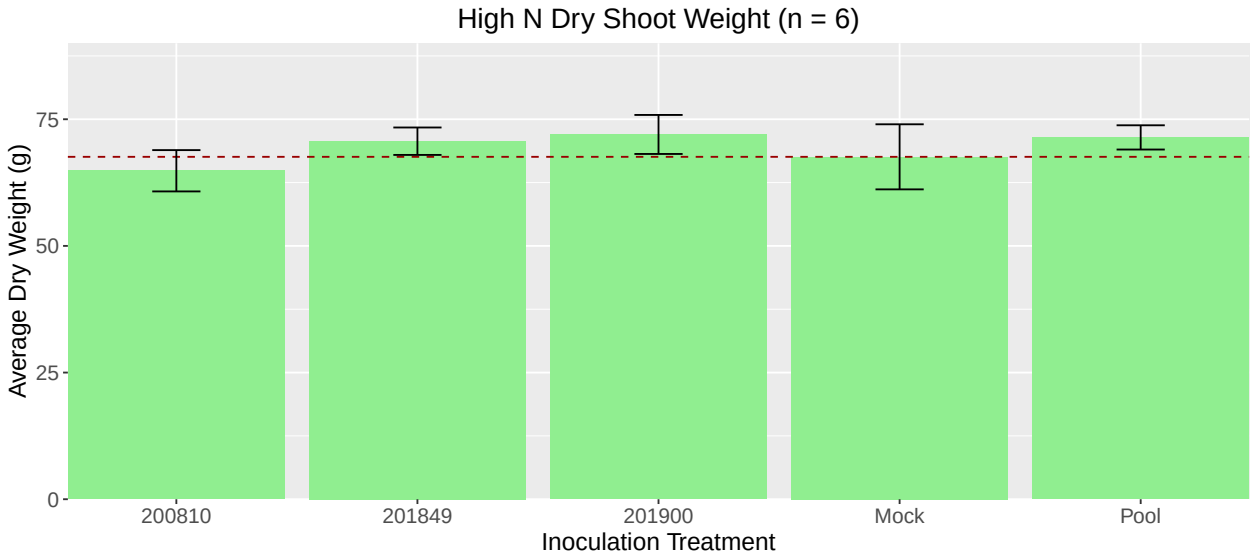


Based on the **reduced experimental data with 3 of the 5 complete blocks**, the estimated mean dry shoot weight of potato plants subjected to high N treatment while receiving inoculations

with isolates 201900, 201849 and the pool is higher than that of the mock inoculated control plants. However, our confidence in the mean estimate difference is low and the effect size is relatively small.

Bar Plot

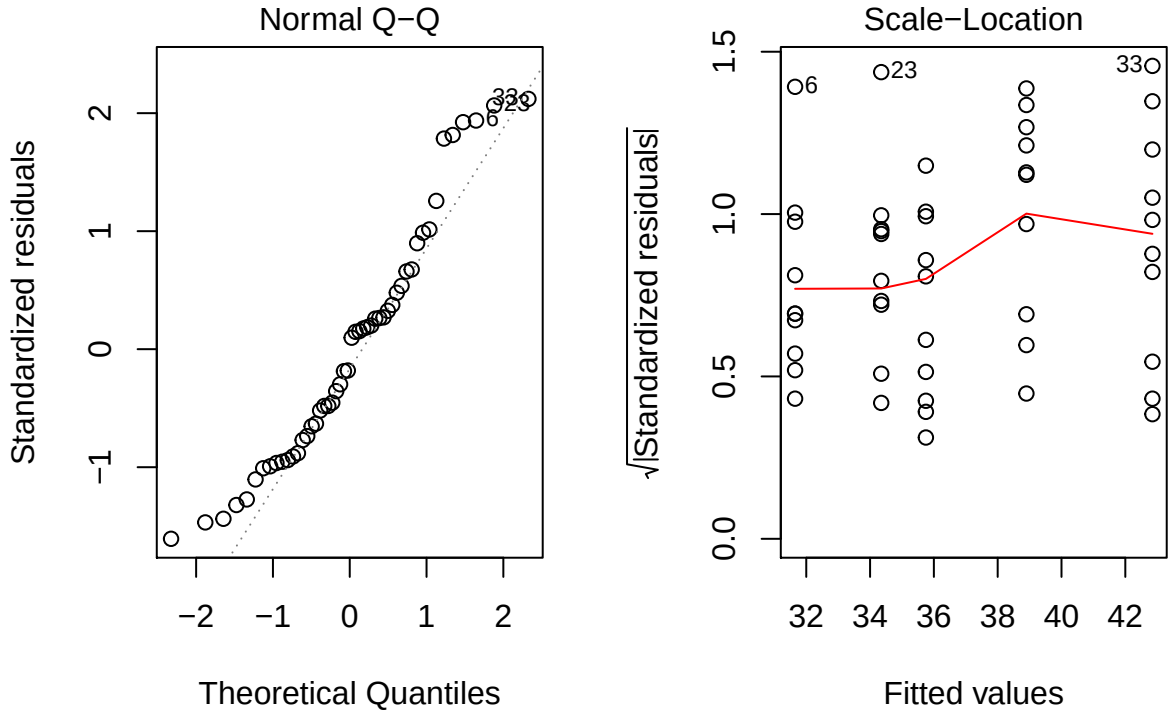
n=6



There are no observed differences of interest for shoot weight among treatment groups under high nitrogen fertilization.

Root Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: root_dry_wt_g
##           Df Sum Sq Mean Sq F value  Pr(>F)
## inoc       4 2736.8   684.2  2.4488 0.07249 .
## Residuals 25 6985.1   279.4
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimated Marginal Means Analysis

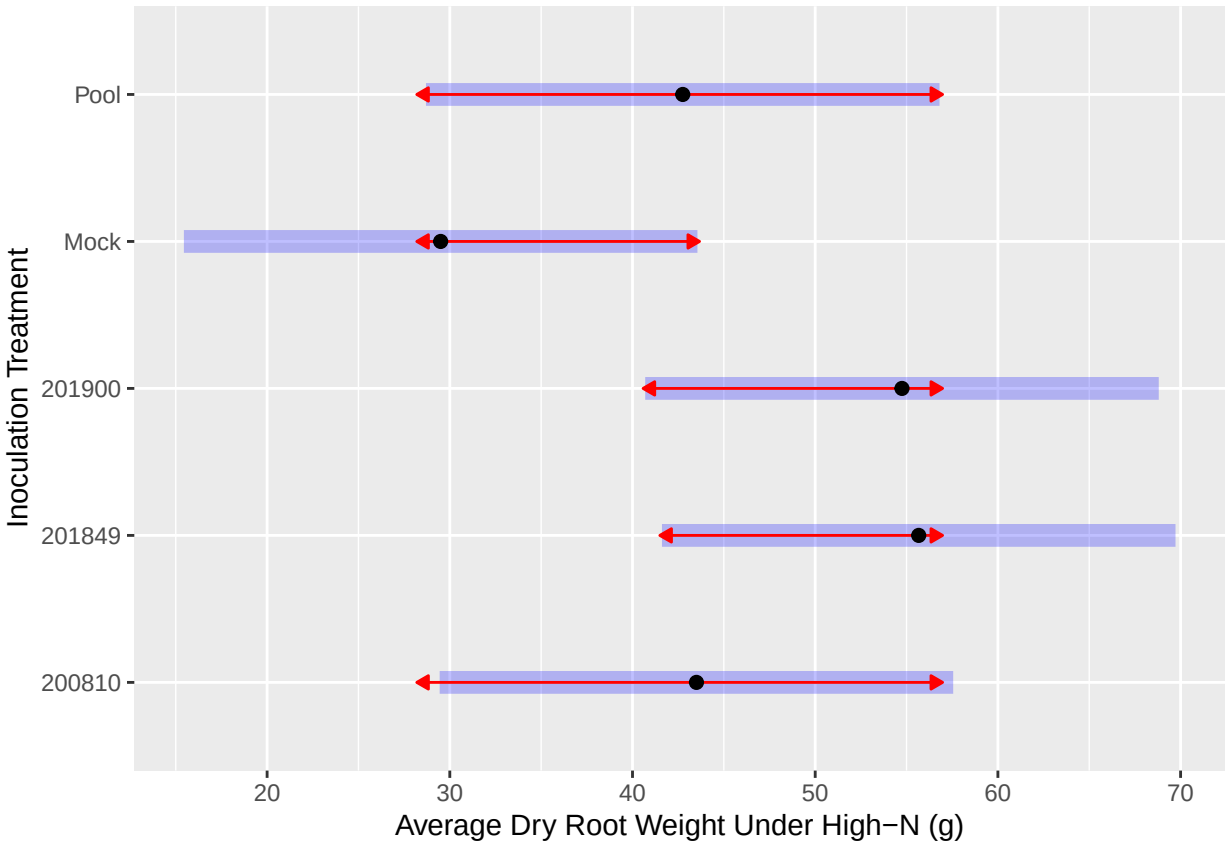
All Pair-wise Comparisons

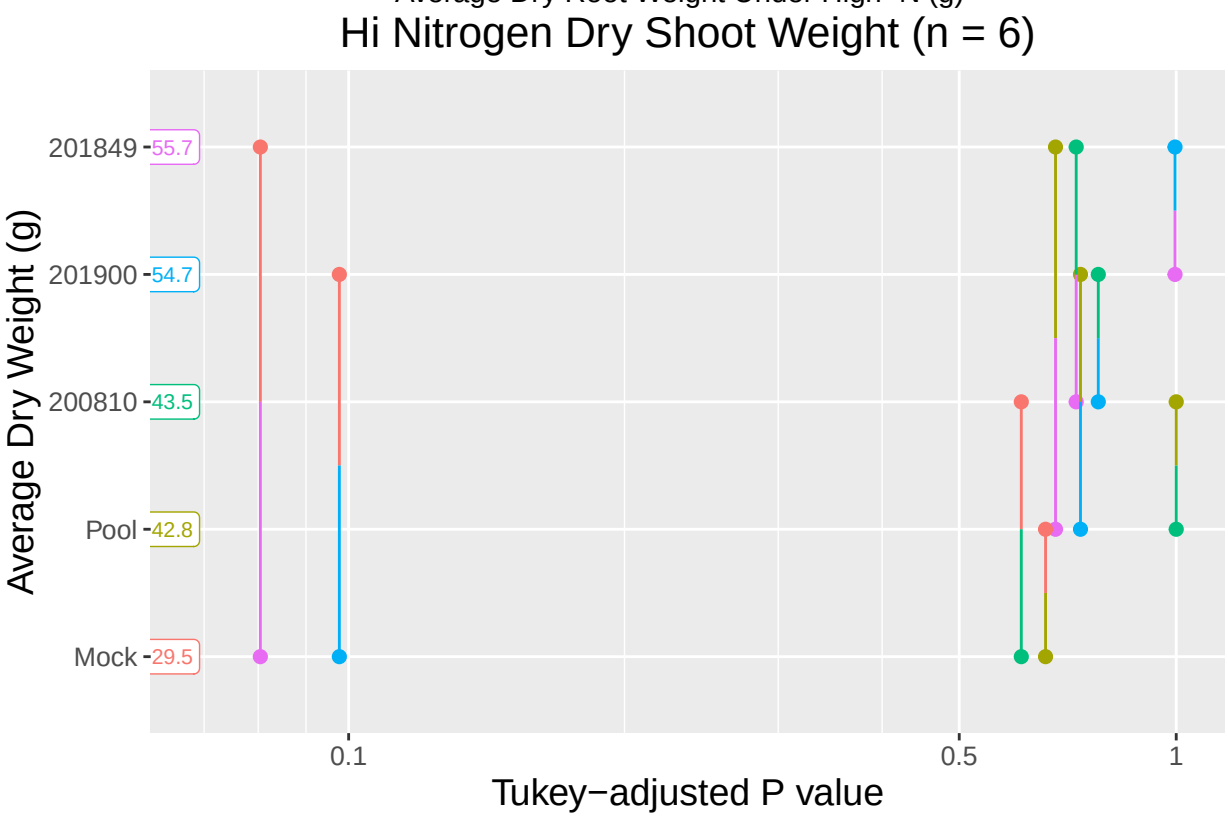
Tukey method of correction for multiple testing

- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849  -12.167 9.65 25 -1.261  0.7167
##  200810 - 201900  -11.250 9.65 25 -1.166  0.7702
##  200810 - Mock     14.000 9.65 25  1.451  0.6022
##  200810 - Pool      0.750 9.65 25  0.078  1.0000
##  201849 - 201900    0.917 9.65 25  0.095  1.0000
##  201849 - Mock     26.167 9.65 25  2.711  0.0805
##  201849 - Pool     12.917 9.65 25  1.338  0.6707
##  201900 - Mock     25.250 9.65 25  2.616  0.0976
##  201900 - Pool     12.000 9.65 25  1.243  0.7267
##  Mock - Pool      -13.250 9.65 25 -1.373  0.6498
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```



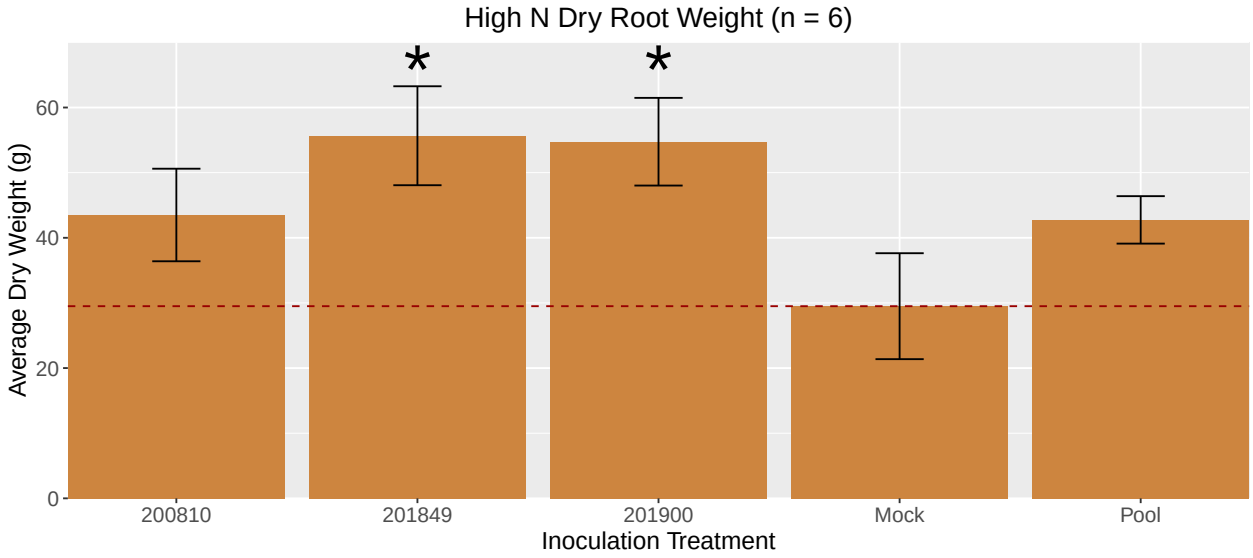


Mean root weights are higher for all inoculated plants relative to mock-inoculated controls. The confidence levels in comparisons of the mean root weight for 201900- and 201849-inoculated plants

with mock-inoculated plants are higher than those for comparisons of controls to 200810- and Pool-inoculated plants.

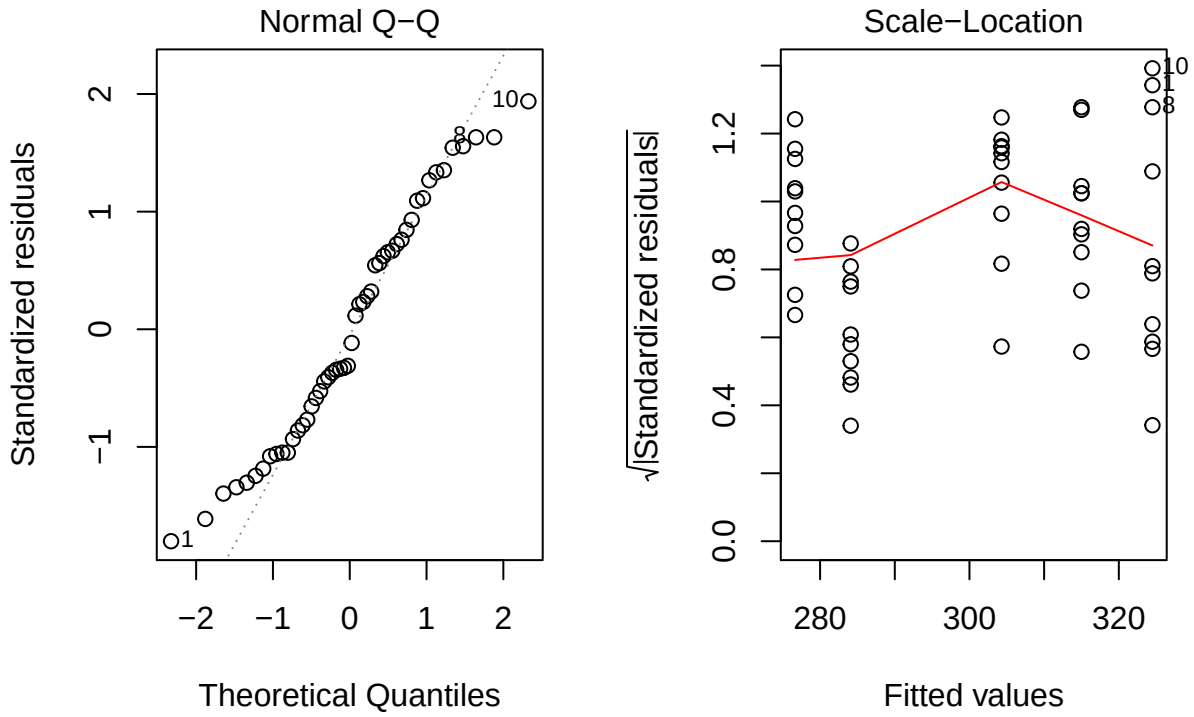
Bar Plot

n=10



Tuber Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
```

```
## Response: tuber_wt_g
##           Df Sum Sq Mean Sq F value Pr(>F)
## inoc       4  23156  5788.9  1.0751 0.3898
## Residuals 25 134608  5384.3
```

Estimated Marginal Means Analysis

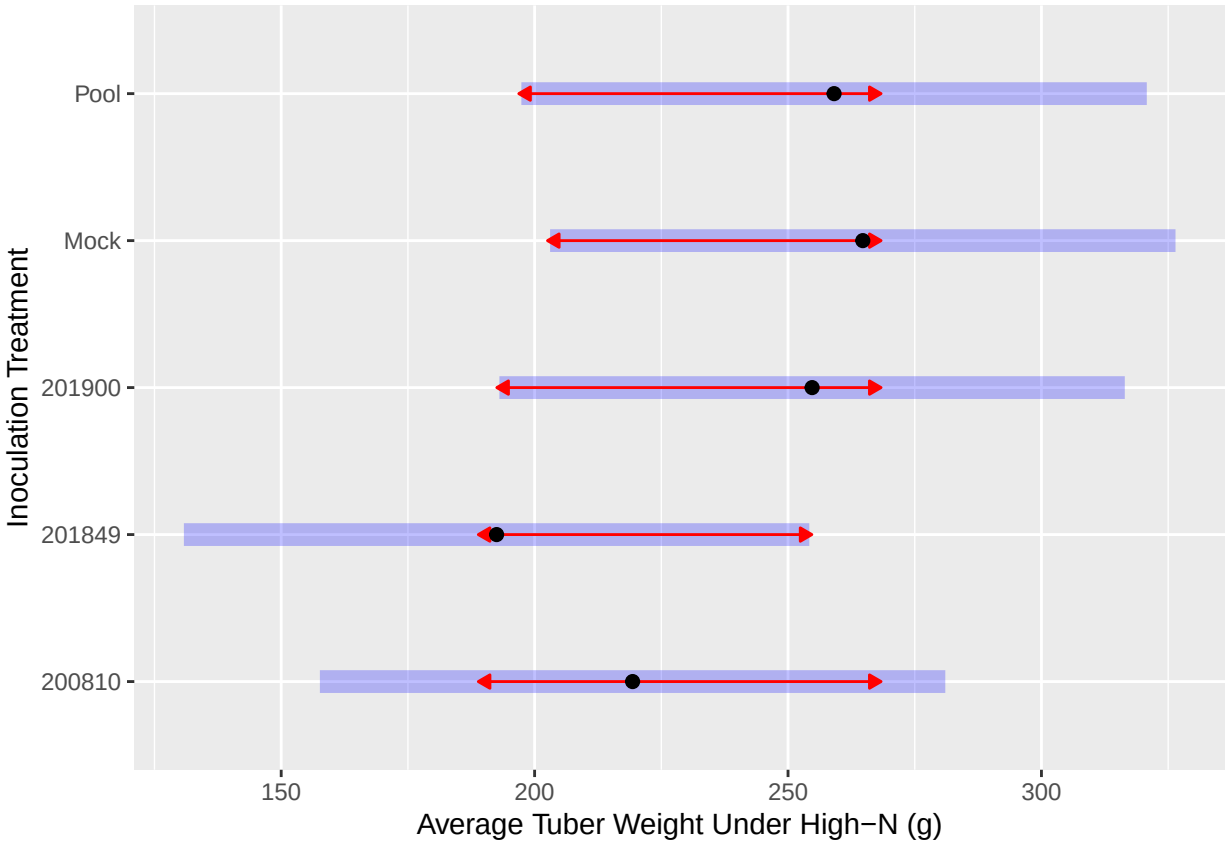
All Pair-wise Comparisons

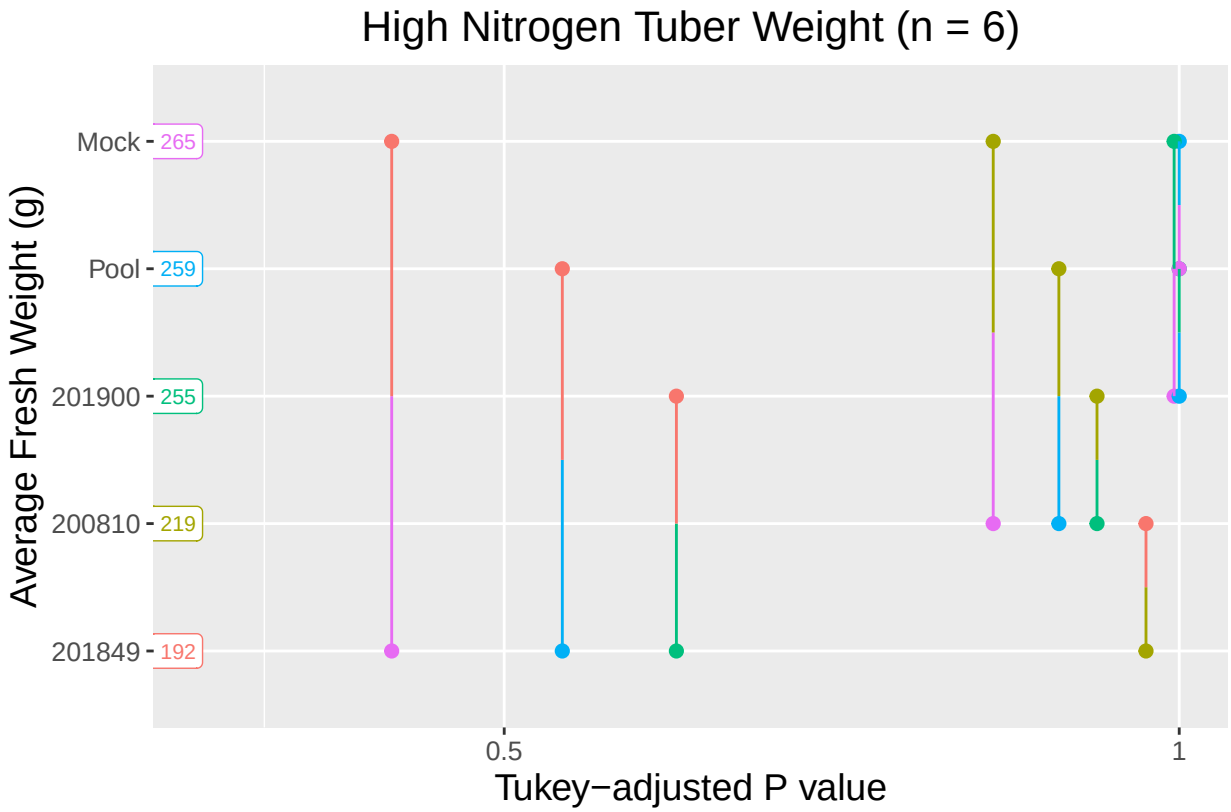
Tukey method of correction for multiple testing

- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849    26.83 42.4 25  0.633  0.9681
##  200810 - 201900   -35.42 42.4 25 -0.836  0.9168
##  200810 - Mock     -45.42 42.4 25 -1.072  0.8189
##  200810 - Pool     -39.75 42.4 25 -0.938  0.8792
##  201849 - 201900   -62.25 42.4 25 -1.469  0.5908
##  201849 - Mock     -72.25 42.4 25 -1.705  0.4488
##  201849 - Pool     -66.58 42.4 25 -1.572  0.5282
##  201900 - Mock     -10.00 42.4 25 -0.236  0.9993
##  201900 - Pool      -4.33 42.4 25 -0.102  1.0000
##  Mock - Pool         5.67 42.4 25  0.134  0.9999
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

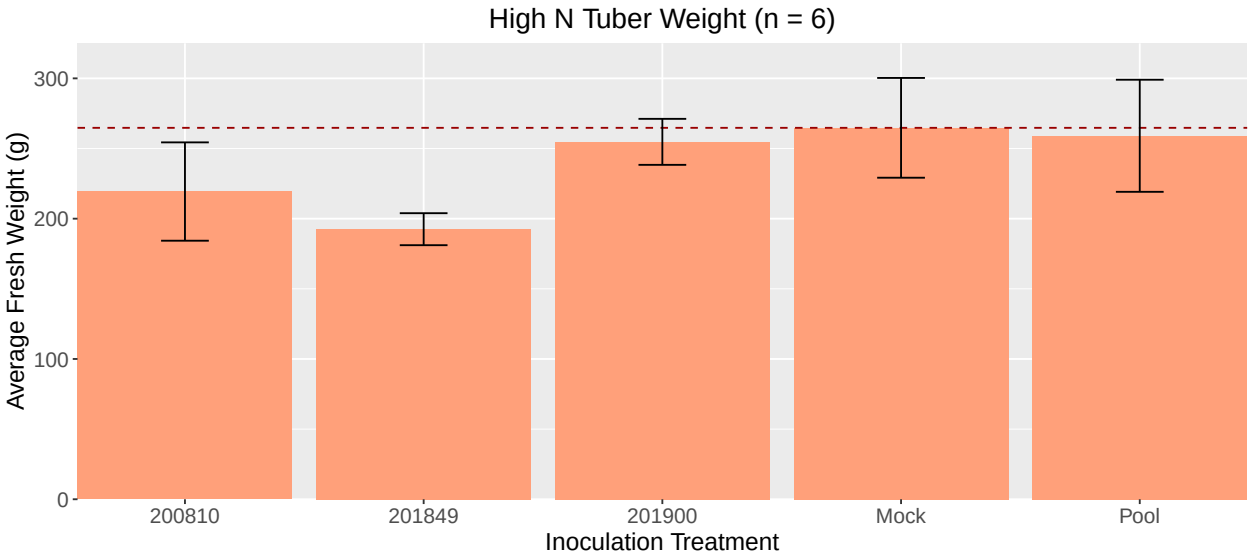




P-values for all pairwise comparisons among treatment groups are high for this response variable. Therefore confidence in the estimated differences based on the dataset is hi.

Bar Plot

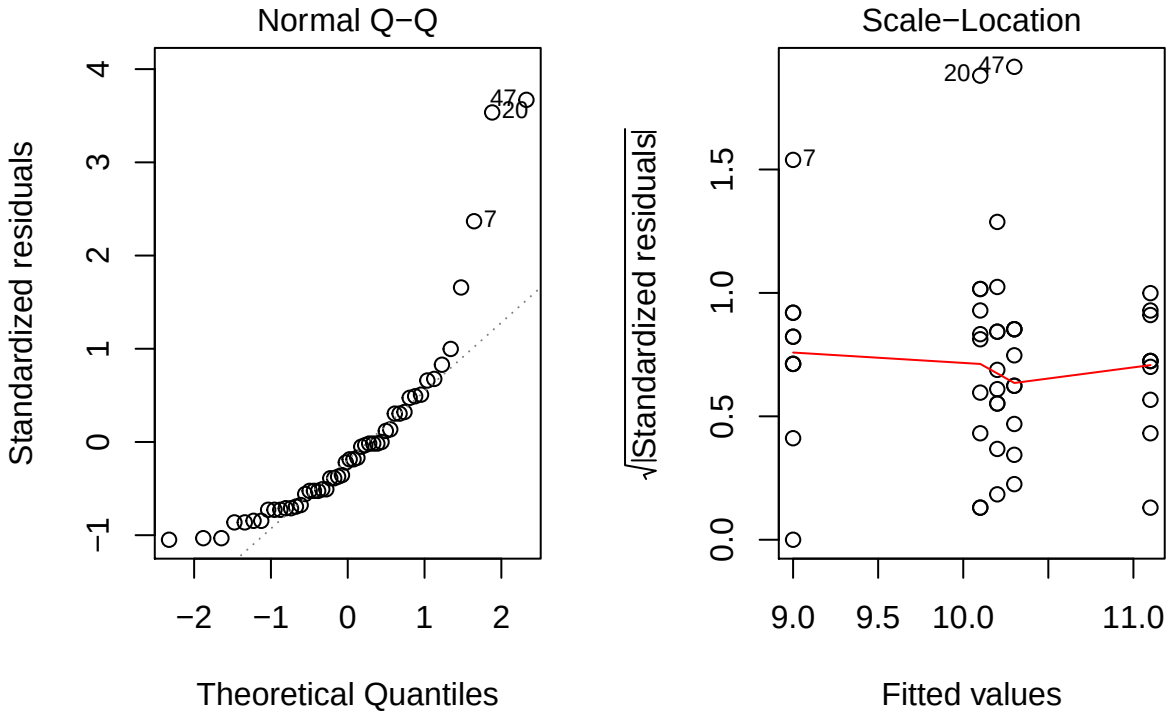
n=10



While the average tuber weight for 201900 inoculated plants is higher than that of the Mock-inoculated group, the confidence in the estimated difference is hi due to high variance in the dataset.

Tuber Number

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: n_tuber
##           Df  Sum Sq Mean Sq F value Pr(>F)
## inoc       4  91.133 22.7833  2.3312 0.0836 .
## Residuals 25 244.333  9.7733
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

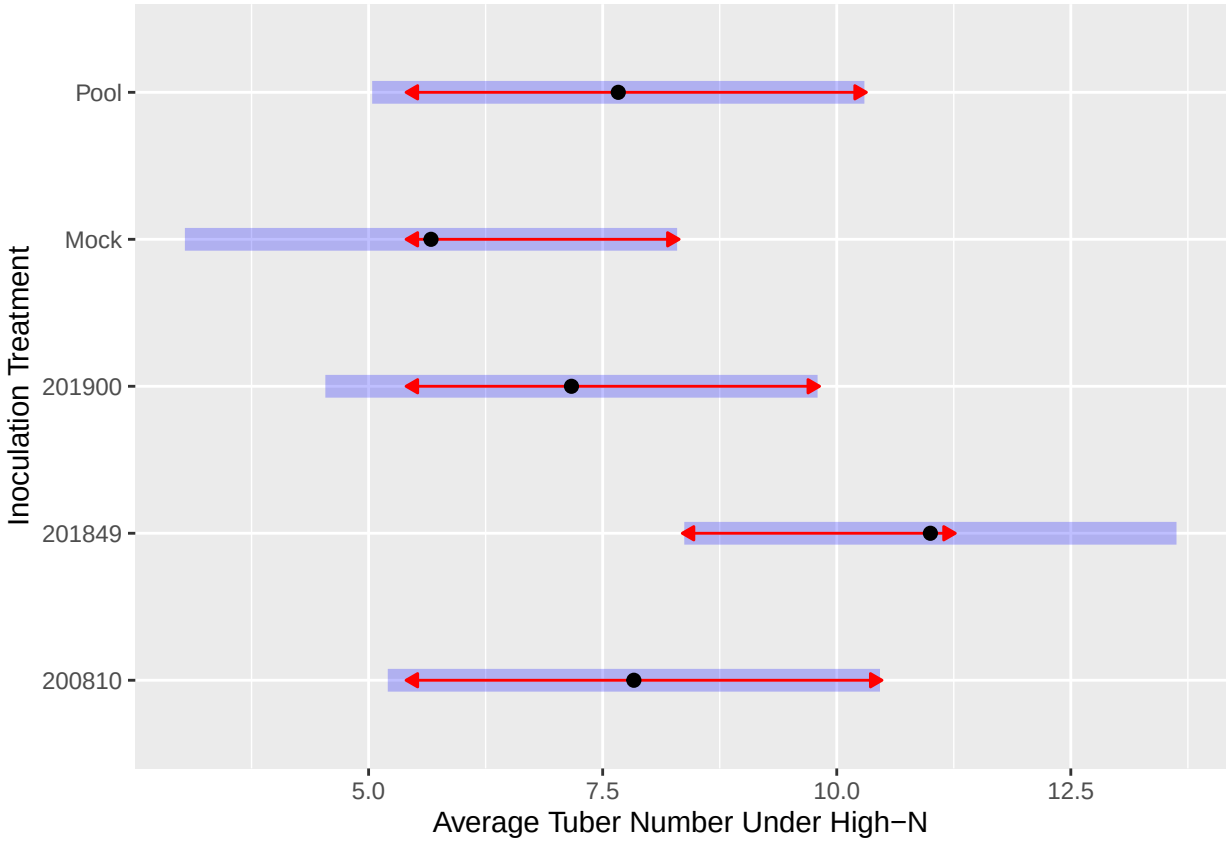
Tukey method of correction for multiple testing

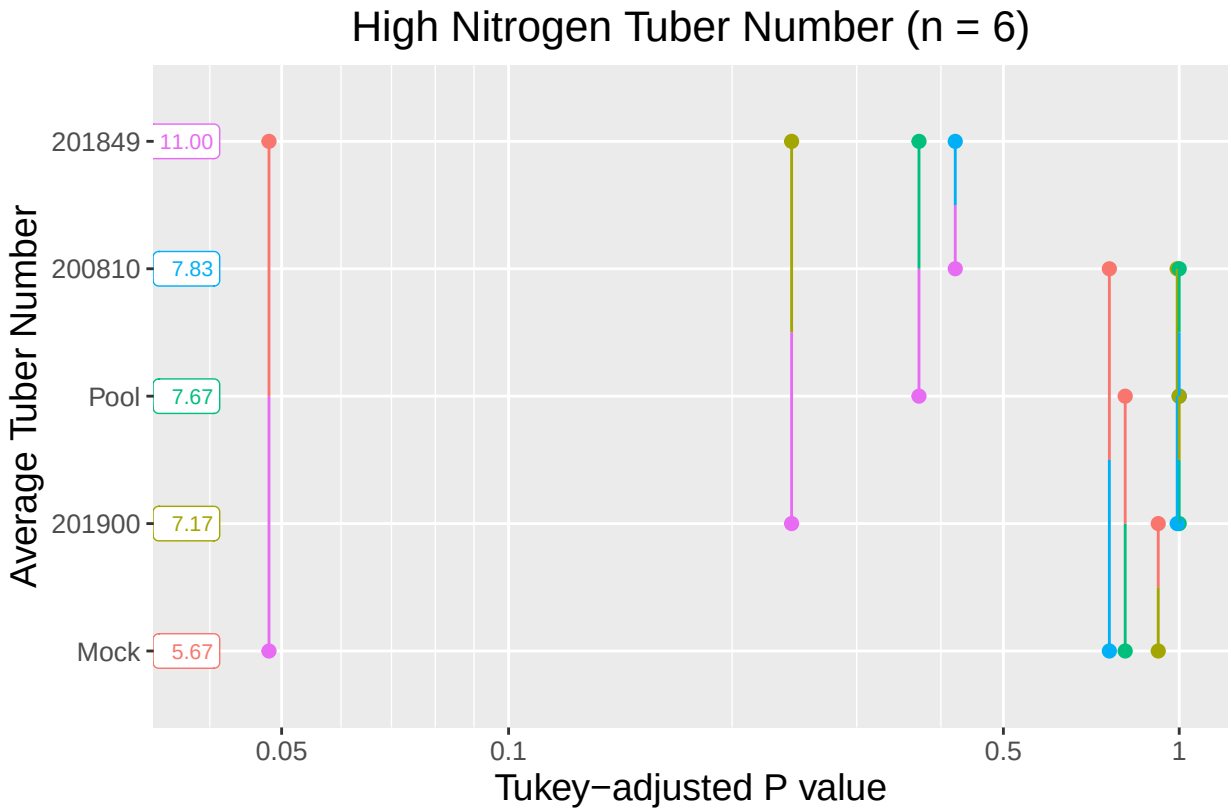
- Alpha Level = 0.05

```
##  contrast        estimate  SE df t.ratio p.value
##  200810 - 201849   -3.167 1.8 25 -1.754  0.4209
##  200810 - 201900    0.667 1.8 25  0.369  0.9958
##  200810 - Mock      2.167 1.8 25  1.200  0.7511
##  200810 - Pool      0.167 1.8 25  0.092  1.0000
##  201849 - 201900    3.833 1.8 25  2.124  0.2417
##  201849 - Mock      5.333 1.8 25  2.955  0.0481
##  201849 - Pool      3.333 1.8 25  1.847  0.3707
##  201900 - Mock      1.500 1.8 25  0.831  0.9184
##  201900 - Pool     -0.500 1.8 25 -0.277  0.9986
##  Mock - Pool       -2.000 1.8 25 -1.108  0.8007
##
```

```
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

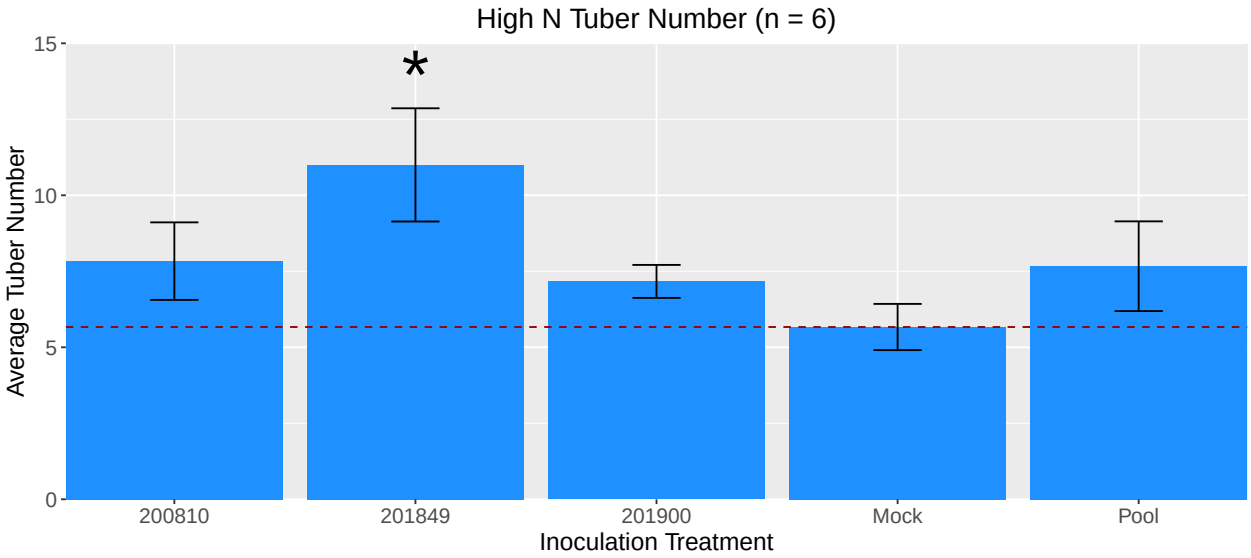




Based on these results, 201849 is the most likely causal factor in the doubling of tuber number observed in the previous experiment.

Bar Plot

n=6



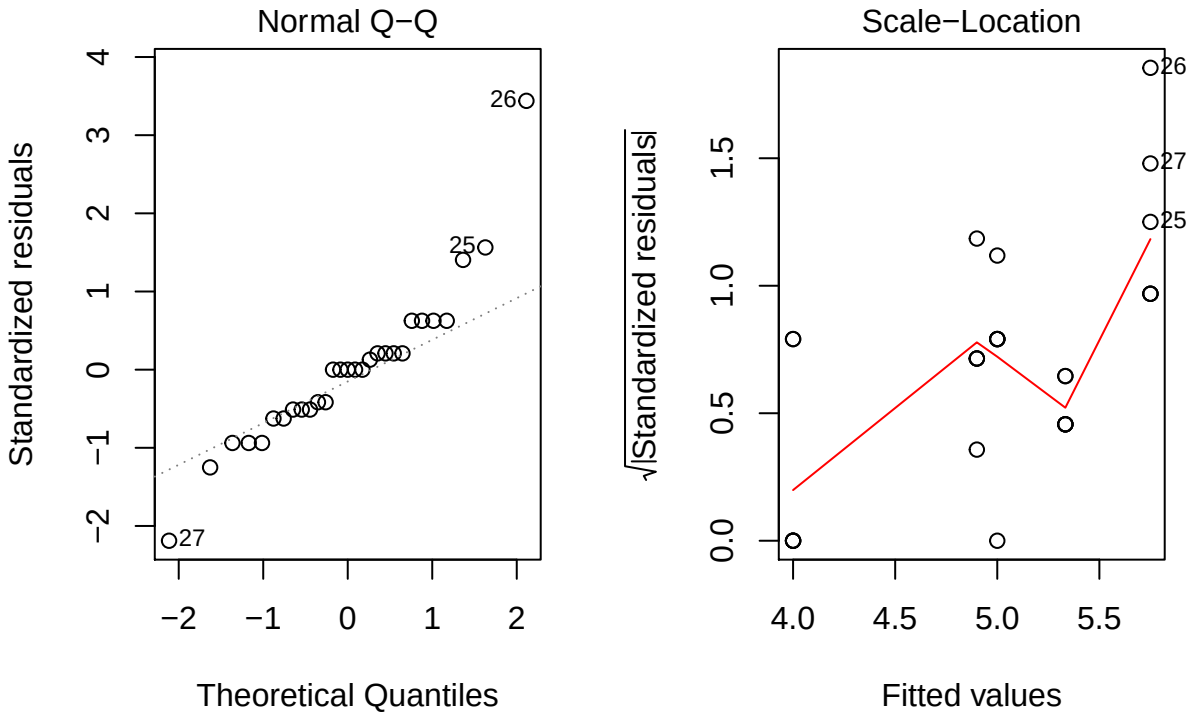
Tuber number increased by 100% with 201849 isolate inoculation, but tuber weight is reduced. Effect on Tuber number is supported statistically but Differences in mean tuber weight is not.

Low Nitrogen

n = 6 for each treatment group

Shoot Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: shoot_dry_wt_g
##           Df Sum Sq Mean Sq F value  Pr(>F)
## inoc       4 10.092 2.52292  3.2893 0.02768 *
## Residuals 24 18.408 0.76701
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Estimated Marginal Means Analysis

All Pair-wise Comparisons

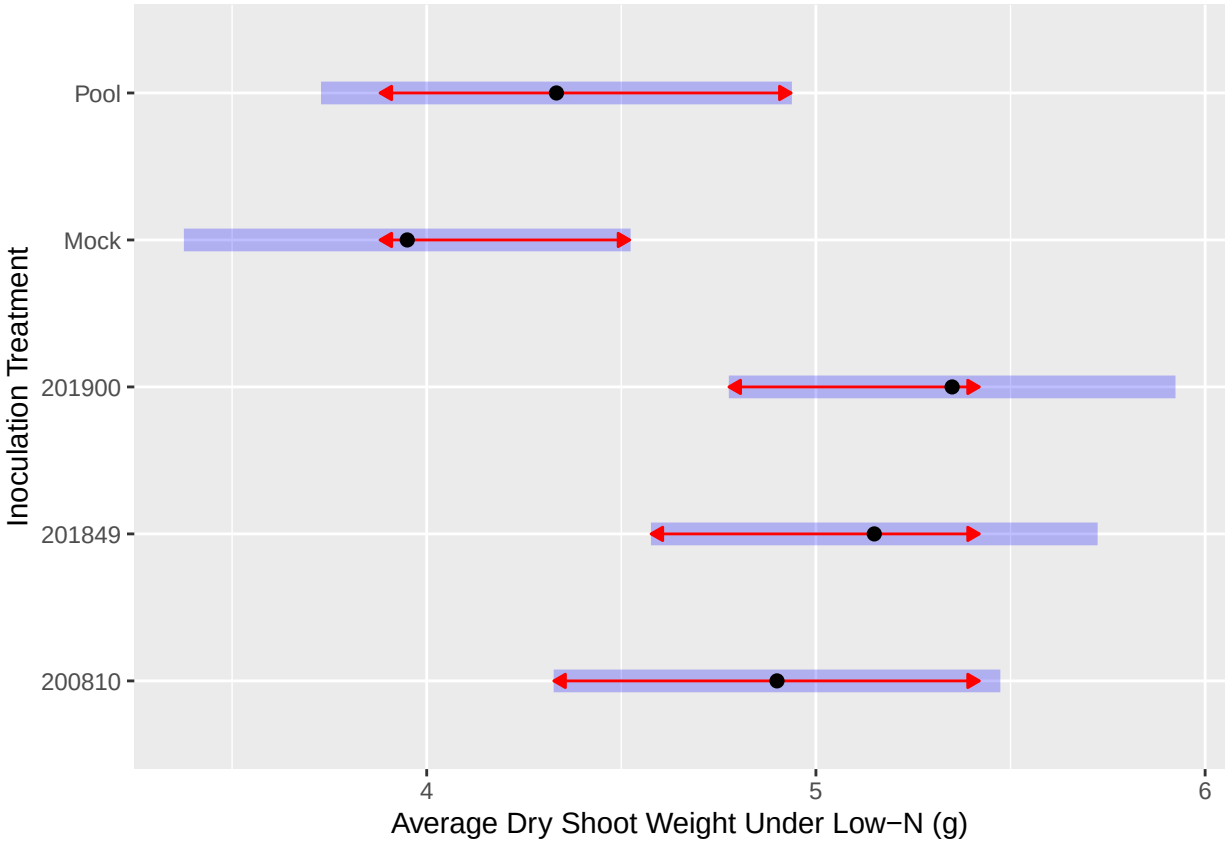
Tukey method of correction for multiple testing

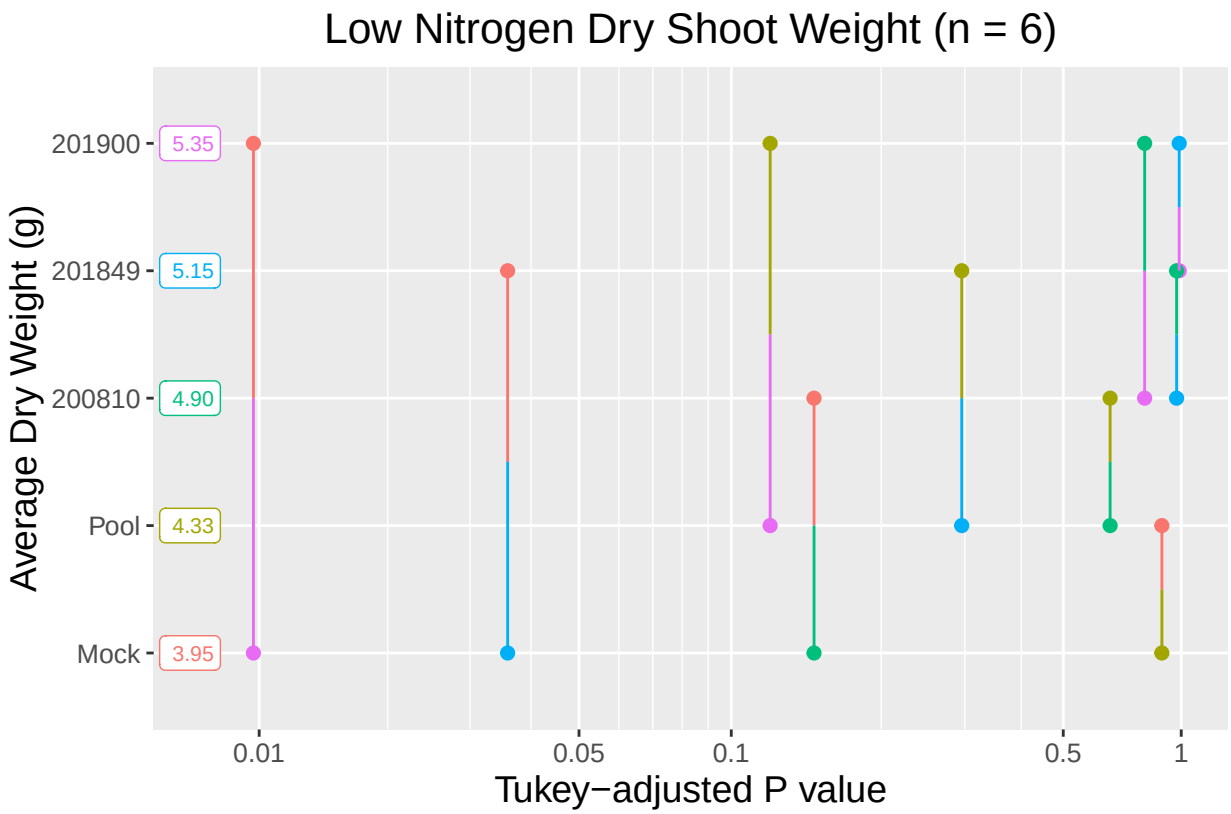
- Alpha Level = 0.05

```
##  contrast        estimate    SE df t.ratio p.value
##  200810 - 201849   -0.250 0.403 44 -0.621  0.9710
##  200810 - 201900   -0.450 0.403 44 -1.117  0.7965
##  200810 - Mock      0.950 0.403 44  2.359  0.1461
##  200810 - Pool      0.567 0.414 44  1.370  0.6498
##  201849 - 201900   -0.200 0.403 44 -0.497  0.9873
##  201849 - Mock      1.200 0.403 44  2.980  0.0359
##  201849 - Pool      0.817 0.414 44  1.974  0.2954
##  201900 - Mock      1.400 0.403 44  3.477  0.0097
```

```
##  201900 - Pool      1.017 0.414 44  2.457  0.1194
##  Mock - Pool       -0.383 0.414 44 -0.927  0.8851
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

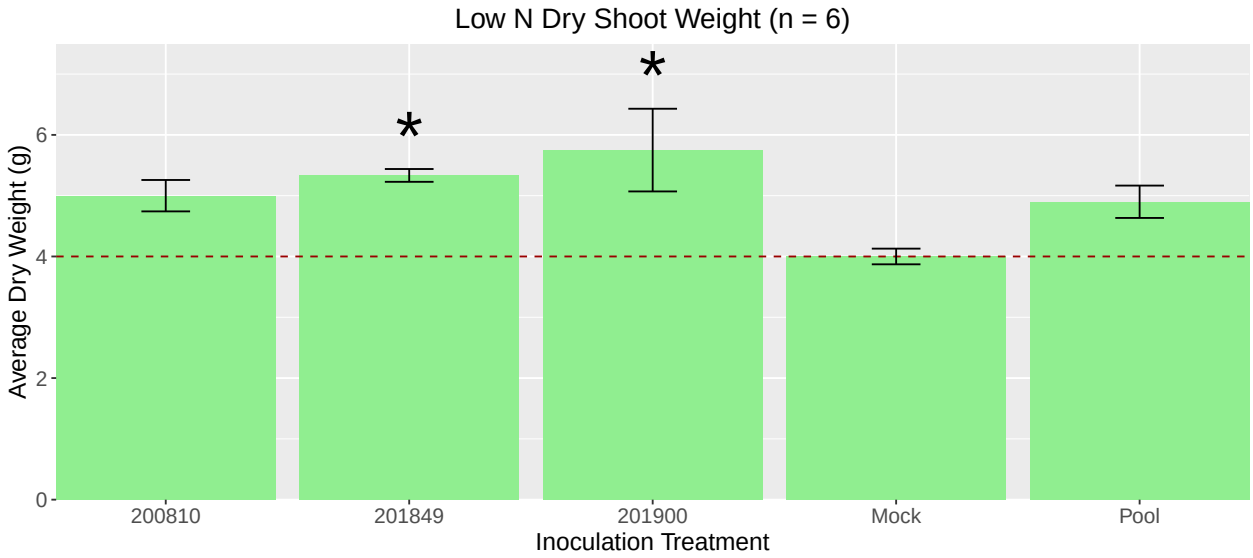




Based on the **reduced experimental data with 3 of the 5 complete blocks**, the estimated mean dry shoot weight of potato plants subjected to low N treatment while receiving mono-isolate inoculations with bacterial isolates is higher than that of the mock inoculated control plants. Plants under low-N that received the inoculation pool of 3 microbes have a mean shoot weight higher than the mock, but the confidence in the mean estimate difference is much lower and the effect size is smaller than that of mono-isolate inoculations. The P-values for the mean estimate comparisons between the mock control plants and those receiving 201849 and 201900 inoculations are both very low (0.05 and 0.01 respectively), which boosts the confidence in the estimated difference in mean shoot weight.

Bar Plot

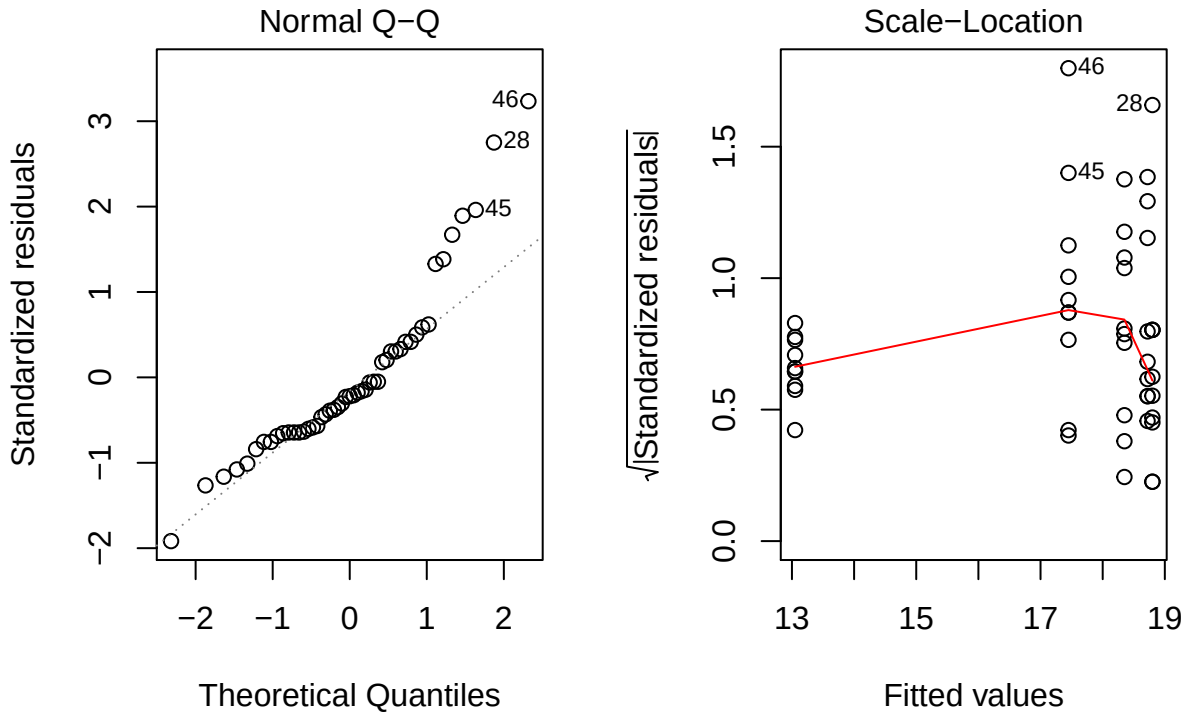
n=6



Increases in shoot biomass were observed for 201849 and 201900 inoculated plants compared to the mock-inoculated plants. The effect size corresponds to roughly a 25% increase in shoot biomass.

Root Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: root_dry_wt_g
##           Df Sum Sq Mean Sq F value Pr(>F)
```

```
## inoc       4 255.20  63.800  1.6597 0.1921
## Residuals 24 922.59  38.441
```

Estimated Marginal Means Analysis

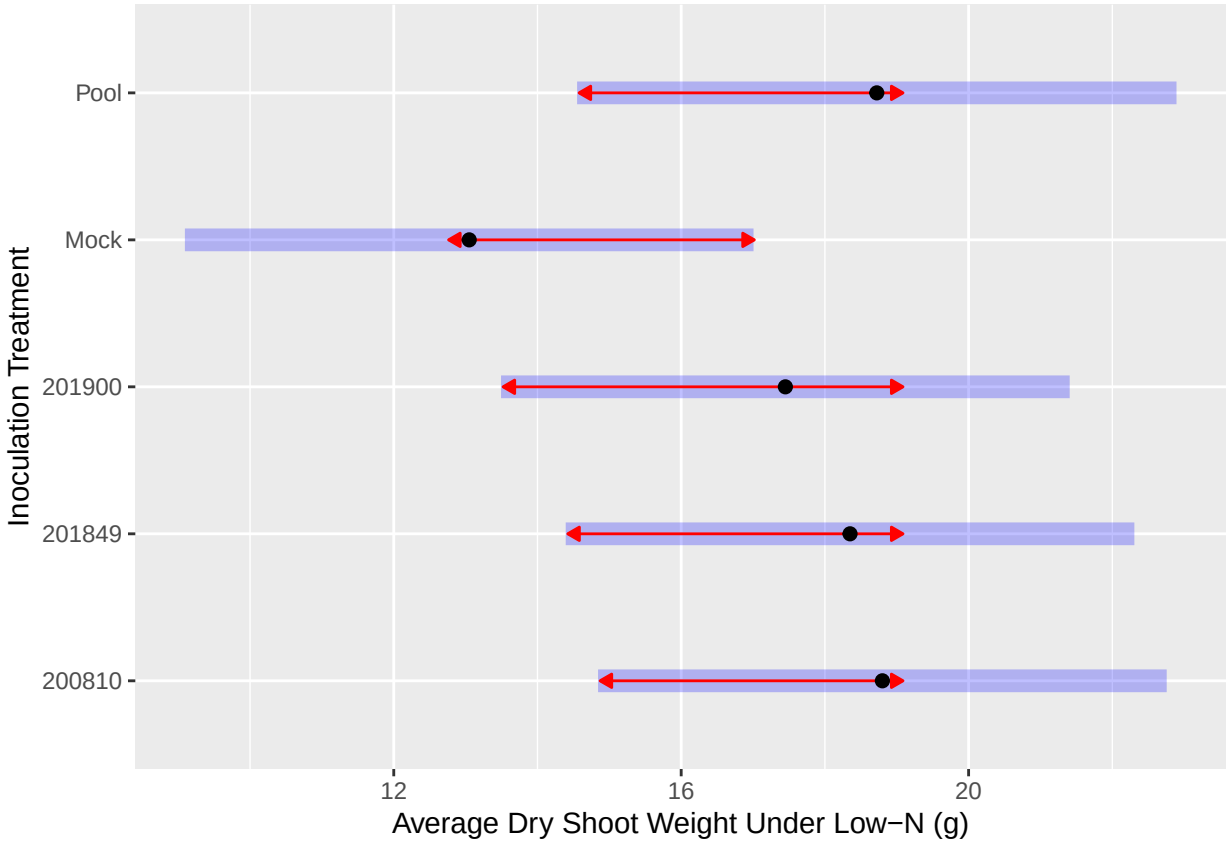
All Pair-wise Comparisons

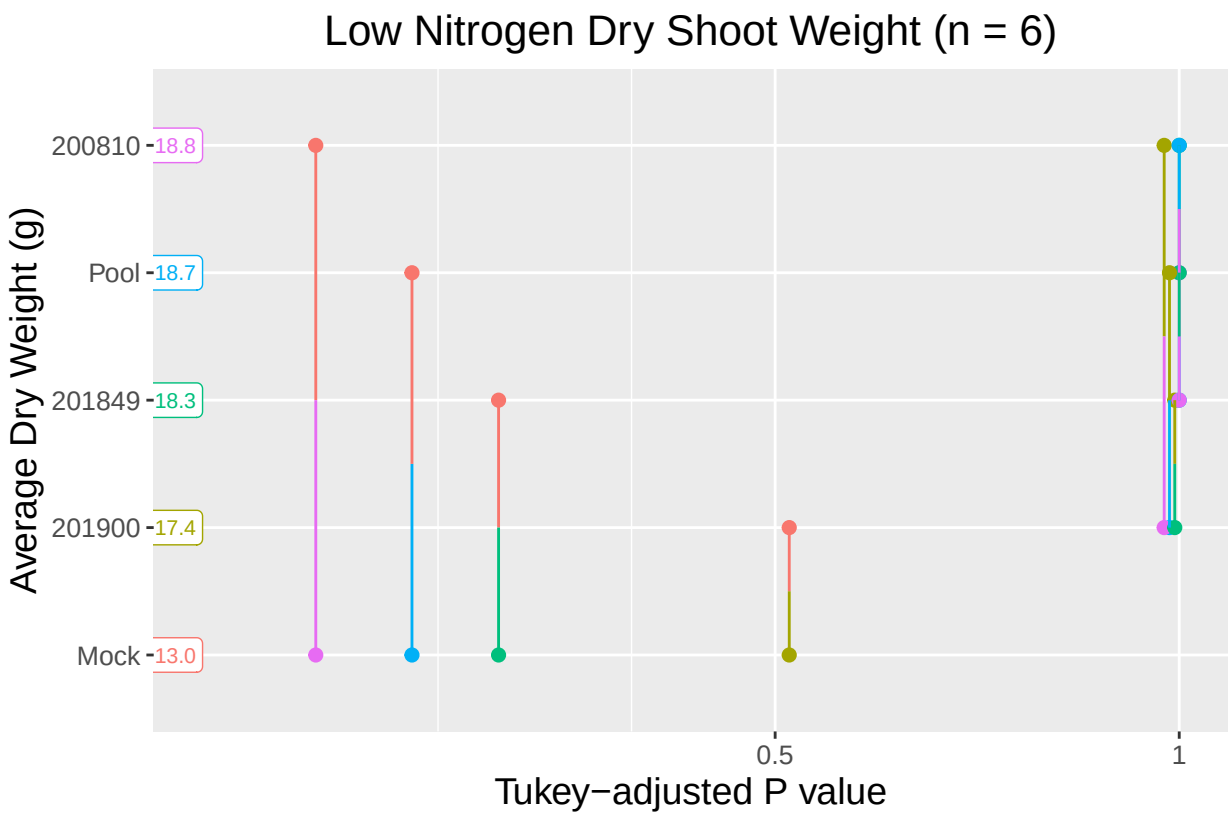
Tukey method of correction for multiple testing

- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849   0.4500 2.78 44  0.162  0.9998
##  200810 - 201900   1.3500 2.78 44  0.486  0.9882
##  200810 - Mock     5.7500 2.78 44  2.071  0.2508
##  200810 - Pool     0.0778 2.85 44  0.027  1.0000
##  201849 - 201900   0.9000 2.78 44  0.324  0.9975
##  201849 - Mock     5.3000 2.78 44  1.909  0.3279
##  201849 - Pool    -0.3722 2.85 44 -0.130  0.9999
##  201900 - Mock     4.4000 2.78 44  1.585  0.5149
##  201900 - Pool    -1.2722 2.85 44 -0.446  0.9915
##  Mock - Pool      -5.6722 2.85 44 -1.988  0.2885
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```

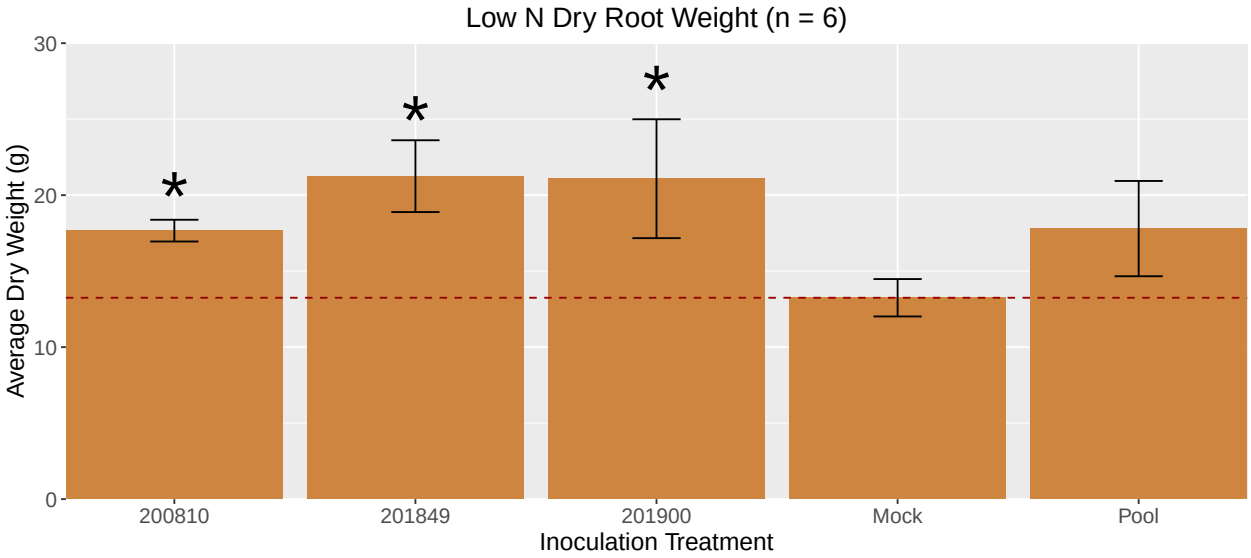




Mean root weights are higher for mono and pool inoculated plants, but the confidence in the means are weak based on statistical analysis of the data. This is due to high variance of the data points for each treatment.

Bar Plot

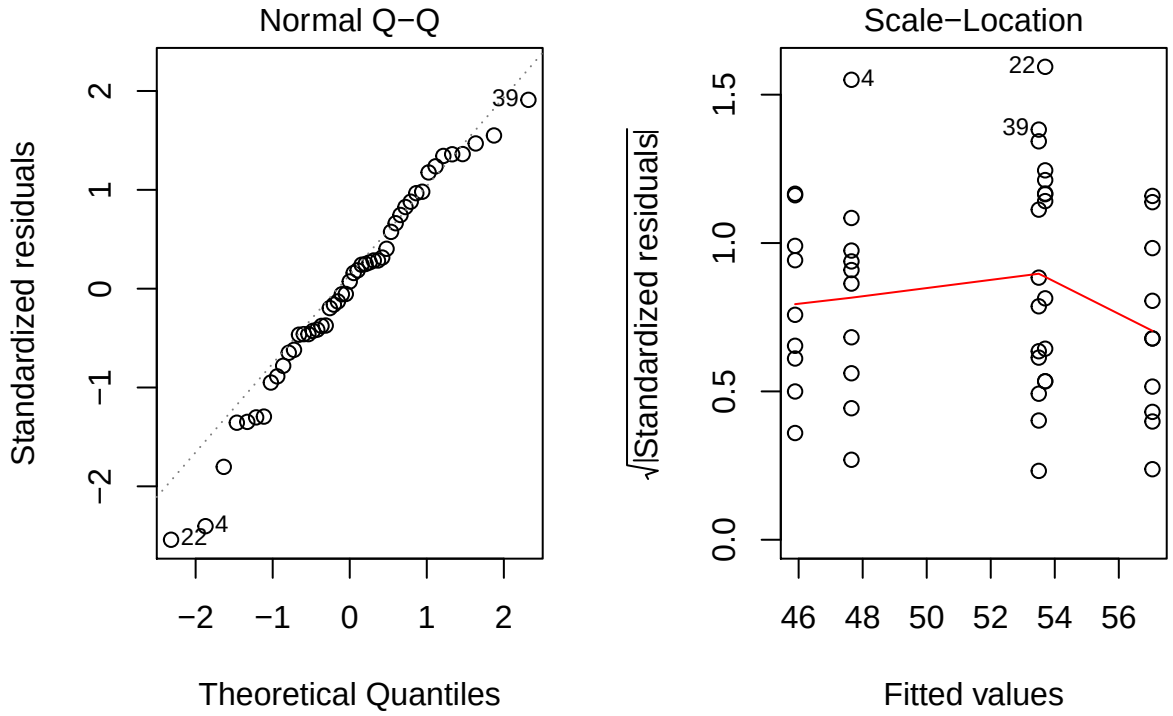
n=10



All microbe-inoculated groups have higher average dry root weights with the reduced dataset, but the condifence levels in the difference comparisons are relatively low based on the P-values above.

Tuber Dry Weight

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: tuber_wt_g
##           Df Sum Sq Mean Sq F value Pr(>F)
## inoc       4 1104.6  276.15  0.7206 0.5863
## Residuals 24 9197.2  383.22
```

Estimated Marginal Means Analysis

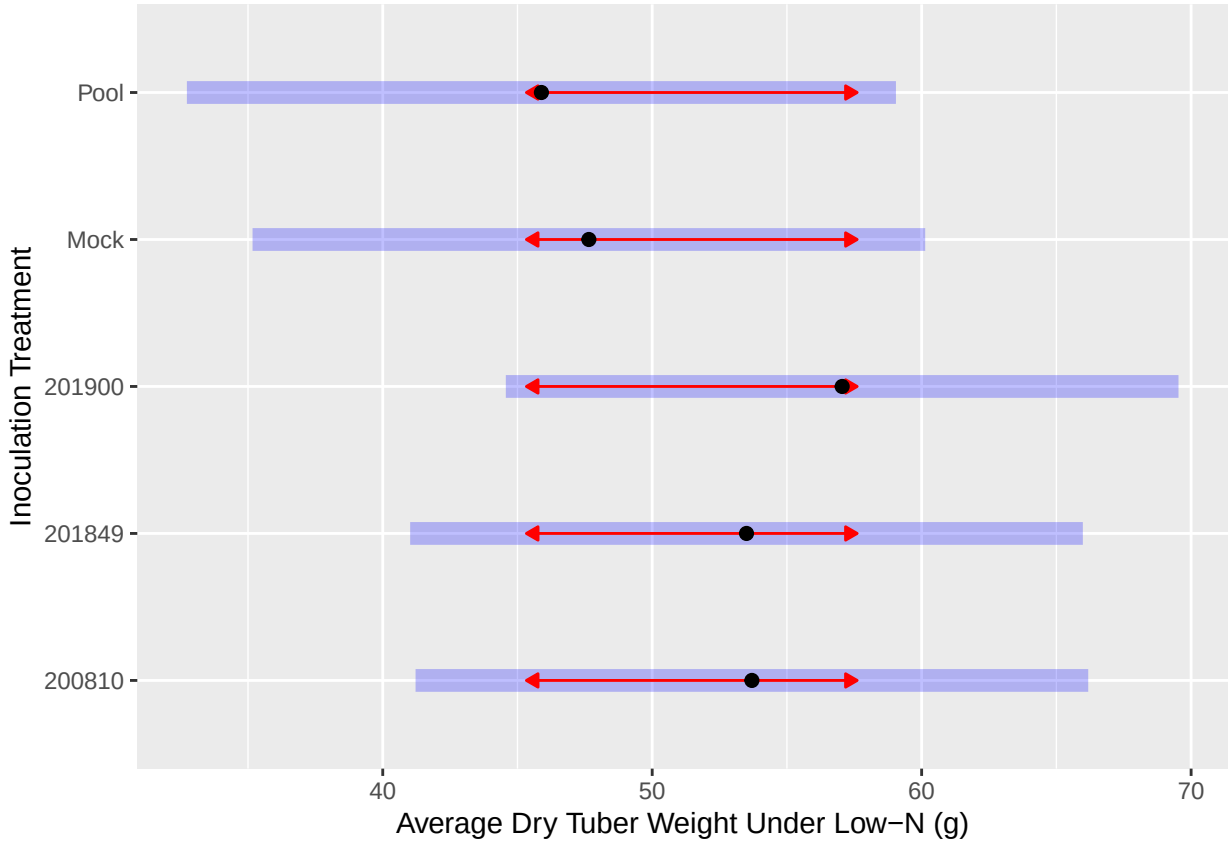
All Pair-wise Comparisons

Tukey method of correction for multiple testing

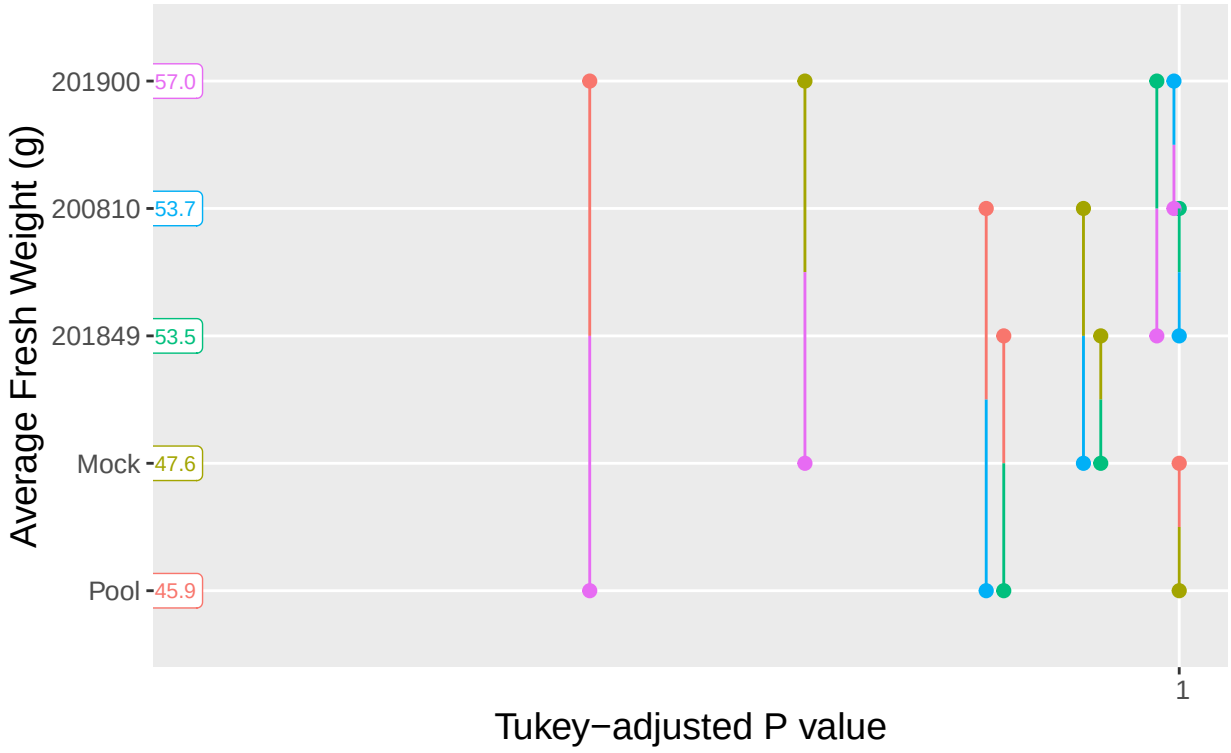
- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849     0.20 8.76 44  0.023  1.0000
##  200810 - 201900    -3.35 8.76 44 -0.382  0.9953
##  200810 - Mock       6.05 8.76 44  0.691  0.9575
##  200810 - Pool       7.81 9.00 44  0.868  0.9070
##  201849 - 201900    -3.55 8.76 44 -0.405  0.9941
##  201849 - Mock       5.85 8.76 44  0.668  0.9622
##  201849 - Pool       7.61 9.00 44  0.846  0.9147
##  201900 - Mock       9.40 8.76 44  1.073  0.8192
##  201900 - Pool      11.16 9.00 44  1.240  0.7281
##  Mock - Pool         1.76 9.00 44  0.196  0.9997
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```



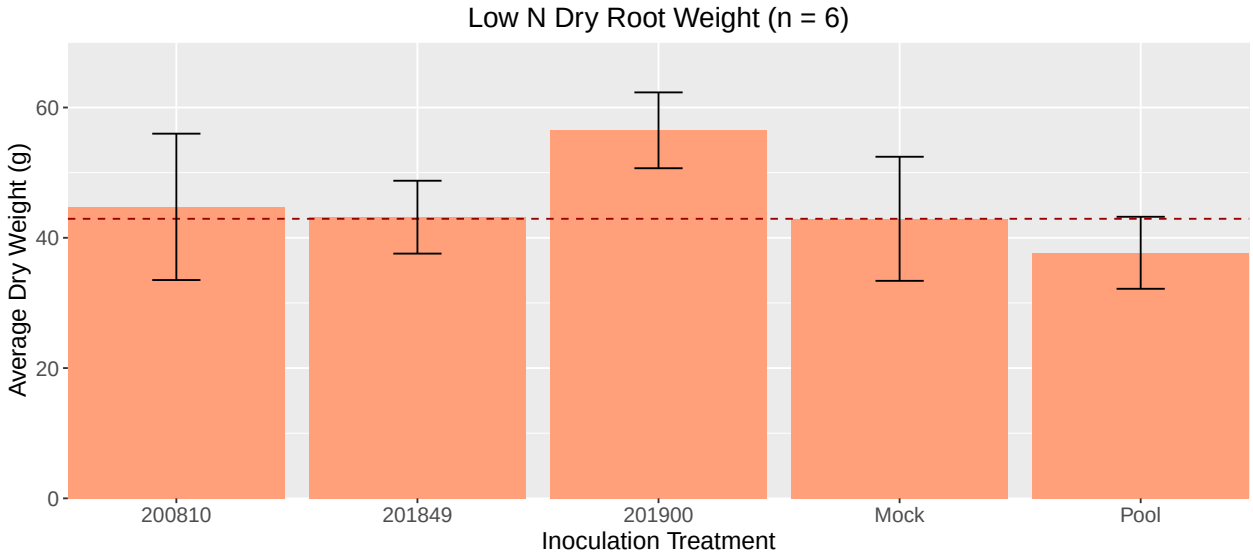
Low Nitrogen Tuber Weight (n = 6)



P-values for all pairwise comparisons among treatment groups are high for this response variable. Therefore confidence in the estimated differences based on the dataset is low.

Bar Plot

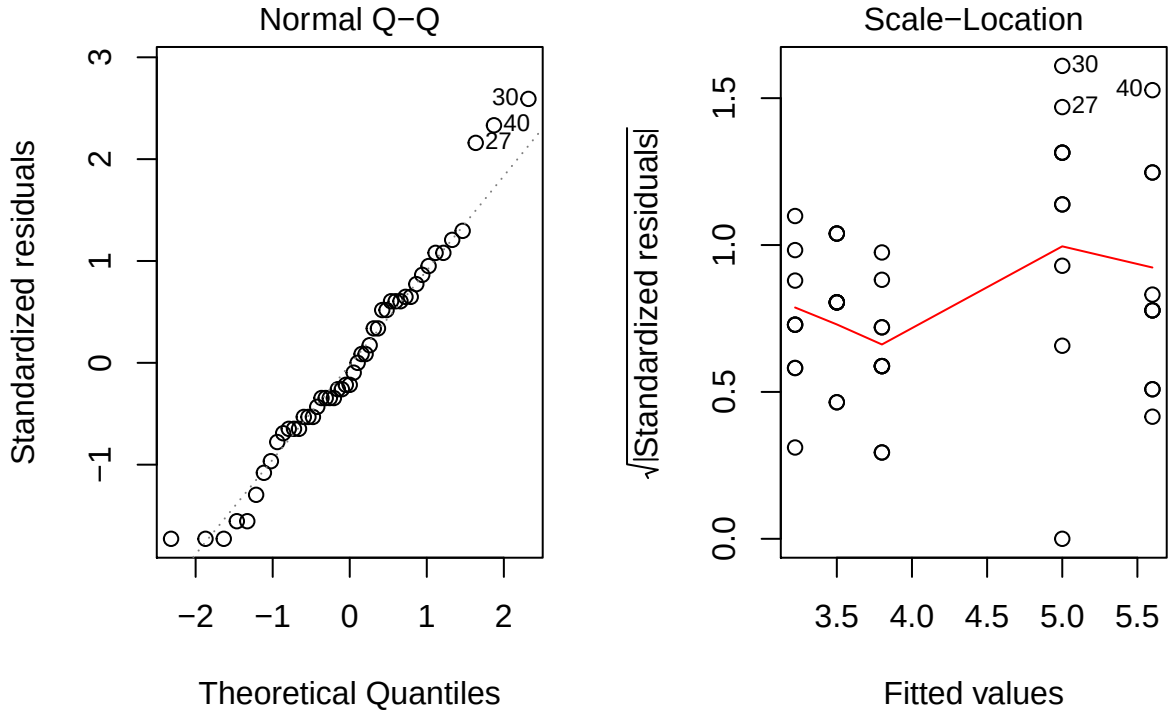
n=10



While the average tuber weight for 201900 inoculated plants is higher than that of the Mock-inoculated group, the confidence in the estimated difference is low due to high variance in the dataset.

Tuber Number

Linear Modeling



Anova

```
## Analysis of Variance Table
##
## Response: n_tuber
##           Df Sum Sq Mean Sq F value Pr(>F)
## inoc       4 19.668  4.9170  1.3507 0.2803
## Residuals 24 87.367  3.6403
```

Estimated Marginal Means Analysis

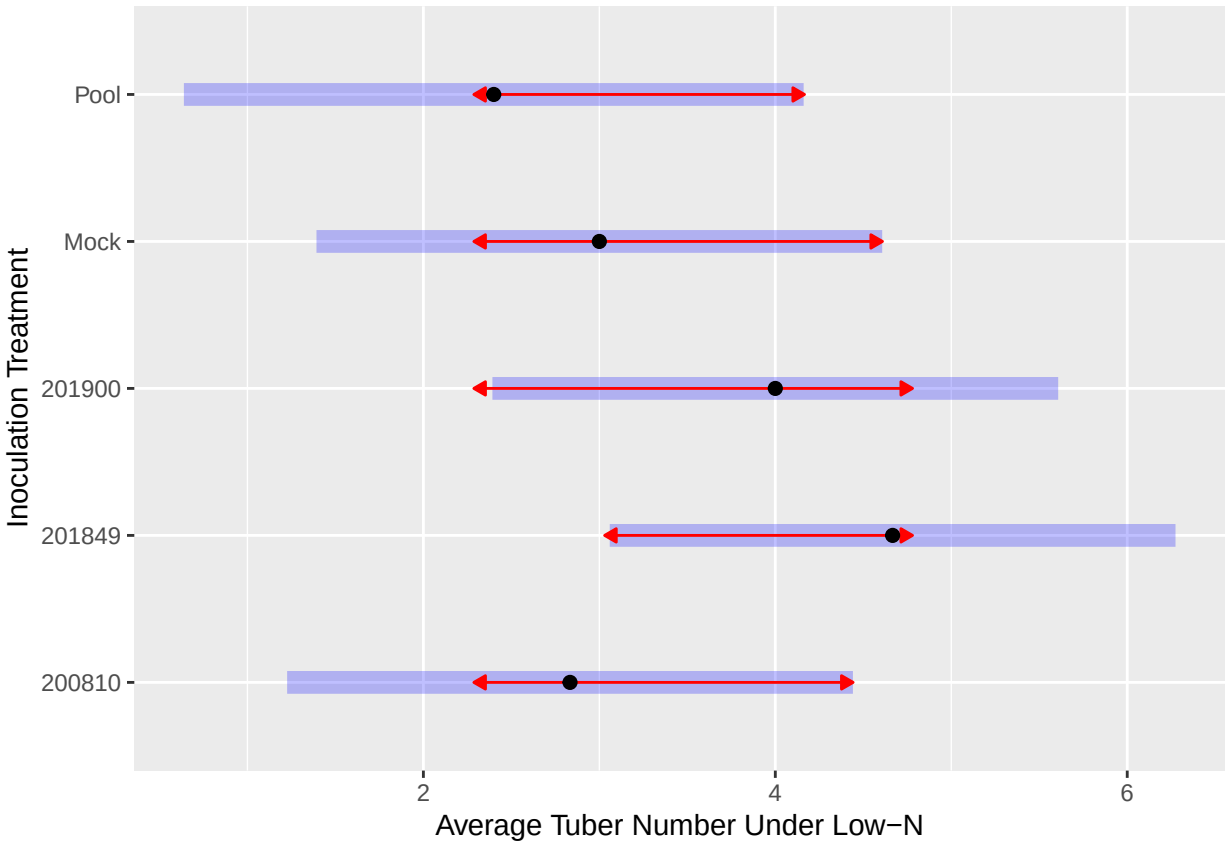
All Pair-wise Comparisons

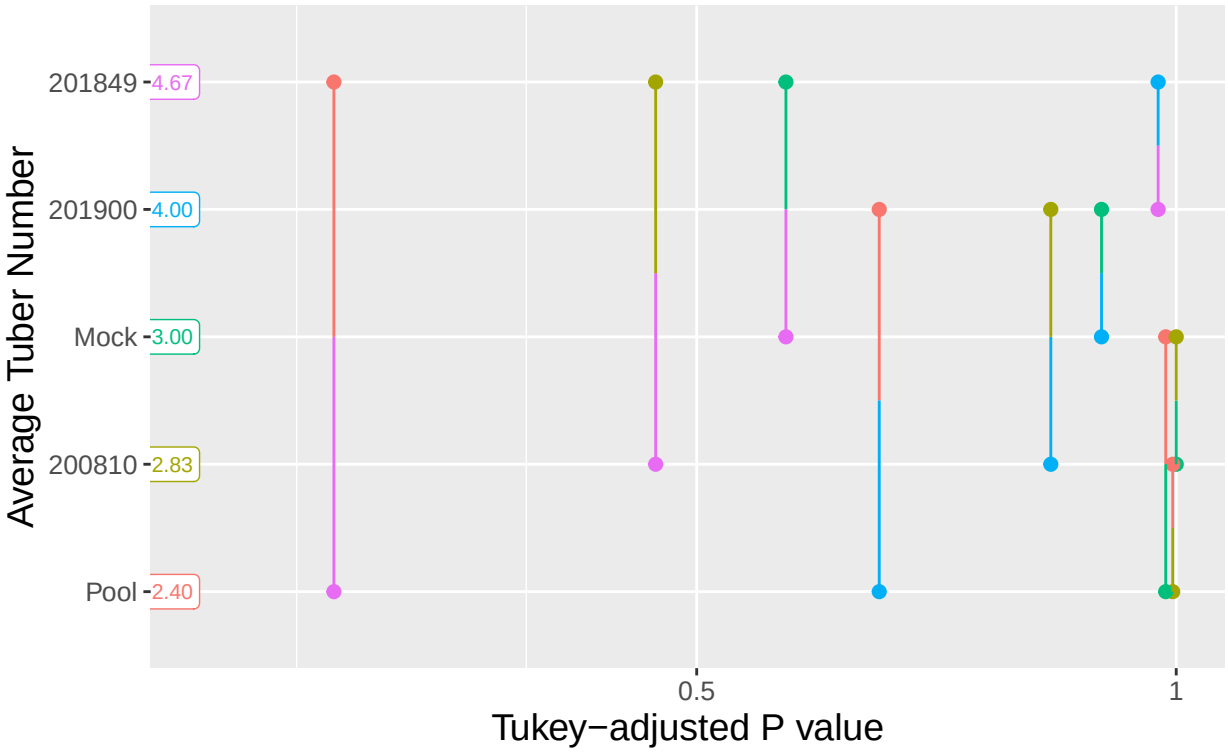
Tukey method of correction for multiple testing

- Alpha Level = 0.05

```
##  contrast        estimate   SE df t.ratio p.value
##  200810 - 201849   -1.833 1.10 24 -1.664  0.4734
##  200810 - 201900   -1.167 1.10 24 -1.059  0.8252
##  200810 - Mock     -0.167 1.10 24 -0.151  0.9999
##  200810 - Pool      0.433 1.16 24  0.375  0.9955
##  201849 - 201900    0.667 1.10 24  0.605  0.9729
##  201849 - Mock      1.667 1.10 24  1.513  0.5644
##  201849 - Pool      2.267 1.16 24  1.962  0.3139
##  201900 - Mock      1.000 1.10 24  0.908  0.8911
##  201900 - Pool      1.600 1.16 24  1.385  0.6427
##  Mock - Pool        0.600 1.16 24  0.519  0.9845
##
## P value adjustment: tukey method for comparing a family of 5 estimates
```

```
## pdf
##   2
```





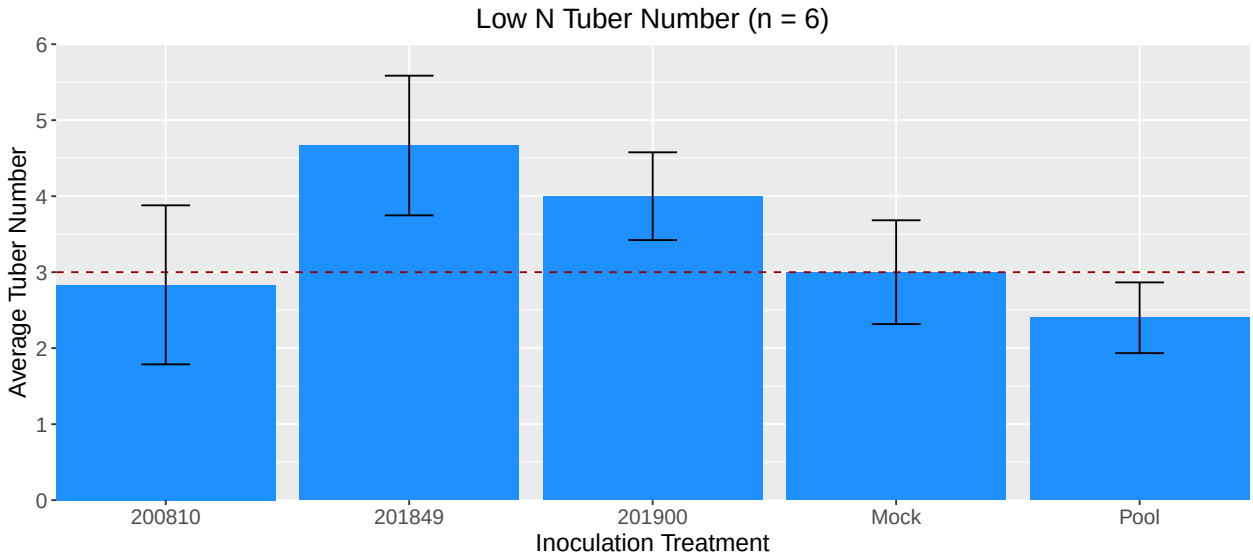
With the reduced dataset, 201849 and 201900 both have higher tuber number than mock-inoculuated plants on average. However, P-values for the comparisons of the differences between

the means are high and lower support for the likelihood that these differences did not occur due to random chance alone.

Compute stats for Bar Plots

n=6

Bar Plot



Analysis with the reduced dataset presents low confidence in the estimated increase in tuber number when plants were inoculated with microbes compared to mock-inoculated plants.

Analytical Summarization

Full Dataset (n = 10)

High Nitrogen

- No significant difference in shoot weight
- No significant difference in root weight
- No significant difference in tuber weight
- No significant difference in tuber number

Low Nitrogen

- 201849 and 201900 induced significant **increase in shoot biomass** compared to mock-control by 25%, or 125% of control
- Microbes appear to increase average root weight, but the confidence in the estimated differences is low.
- No substantial difference in tuber weight among treatment groups with low nitrogen.
- 201849 appears to increase the average number of tubers, but confidence is low and the increase in number does not correspond to an increase in tuber weight (i.e. yield).

Reduced Dataset (n = 6)

High Nitrogen

- There were no observed significant differences in dry shoot weight among the treatment groups.

- A Near doubling of underground root biomass accumulation was observed for 201849 and 201900 inoculated plants compared to mock-inoculated control plants. These contrasts in mean estimation for root weight have a high degree of confidence in the likelihood that the difference was due to the inoculation and not random chance alone.
- Mock-inoculated plants had the highest tuber weight in this reduced dataset.
- 201849 induced a doubling of tuber number in the reduced dataset and the contrast has a reasonable confidence level based on the P-value at the alpha level of 0.05. This provides one piece of evidence to suggest that 201849 may be the causal factor for the observed doubling of tuber number that was observed in the previous experiment.

Low Nitrogen

- With the reduced dataset, 201849 and 201900 both increased the shoot weight of the potato plants by roughly 25% compared to the mock-inoculated control plants under low N-fertilization. These differences have a high degree of confidence based on the alpha-level of 0.05 and associated low P-values.
- Underground root biomass was higher for all microbe-inoculated groups compared to mock-inoculated plants, but the confidence levels for the contrasts are low with relatively high P-values.
- Inoculation with 201900 produced the highest average tuber weight with the reduced dataset, but our confidence in the likelihood that the difference occurred based on the inoculation rather than due to random chance is low.
- 201849 had the highest average tuber number, but the confidence level in the difference estimation with the mock-inoculated plant is low based on the high P-value.

Overall, it seems that adding 201849 and 201900 do have some effects on the growth of potato plants, but these effects to do translate to increases in tuber biomass. There are likely hormonal effects occuring with the presence of these microbes in high dosages that impact the development of the potato plant tissues. Increases to udnerground root biomass were observed for High and Low N plants with the reduced dataset, which satisties one of the initial justification criteria to carry out these experiments (support shallow underground roots of potato). In doing this follow up experiment we have acquired evidence that supports the observed doubling in tuber number from the first experiment, where the phenomenon was semi-repeated but not in exactly the same context as in the first experiment. In the first experiment, the doubling effect occured with microbial-pool incoulation whereas in this experiment the doubling was observed with mono-inoculation of isolate 201849. The increase in shoot biomass observed under low nitrogen suggests that providing the microbe to the plant under N-limiting conditions could provide some measurable degree of assistance.