Mid-term Project: ANOVA

# Summary

In this analysis, an unbalanced 3-way analysis of variance was conducted in order to analyze the effects of 3 different factors on plants’ resistance to cold weather. These factors included the species of potato (1 & 2), the temperature they

were previously acclimatized to (cold & room temperature), and the temperature they were later exposed to (-4 degrees celsius & -8 degrees celsius). After discovery of an insignificant 3-way interaction term, 3 main effects were discovered to be significant at the 95% family confidence level, but due to the interactions between the factors, the interpretation of these main effects were limited to specific treatment combinations.

1. The acclimatization regime significantly affected the mean ion leakage of potato species 2. Specifically, those which were acclimatized to cold temperature showed significantly less ion leakage than those acclimatized to room temperature, regardless of the temperature they were later exposed to.
2. Of those potatoes acclimatized only to room temperature, species 1 showed significantly lower ion leakage than species 2, regardless of the temperature they were later exposed to.
3. Of those potatoes acclimatized only to cold temperature, those later subjected to -4 degree celsius temperatures showed significantly lower ion leakage than those subjected to -8 degree celsius temperatures, regardless of the species considered.

While discovery of the third main effect isn’t necessarily useful, the preceding two may be inferentially useful. Species

1 of potatoes fared better than species 2 when both were acclimatized to room temperatures. This may be useful when choosing which plants to cultivate when it is known the climate will be moderate, and further research is indicated with this in mind. More importantly, species 2 exhibited the property of interest, which was acclimatization to cold temperatures increased the species’ resistance to later exposure to both severities of temperatures. Again further research is indicated in determining the properties of species 2 that may allow for this response.

A total of 13 specific pairwise comparisons between the mean ion leakage were found to be significant at the 95% family confidence level, due to the complicated nature of interaction effects, however only the above 3 were contextually meaningful and thus the full summary of individual differences is included in the appendix for the reader to consider (Appendix List 1).

# Key Graphs

Plot (3.1) Ion leakage by species, regime, and temperature

−−

−−

−

−−−

−−

−−

−−

−−

−−−−

−−−−

−−−−

−−−−

Degrees

−4

−8

Degrees

Species1

Species2

Species1

Species2

0

10

20

30

40

50

Potato

Leakage

Regime

Cold

RoomTemp

Grand mean depicted by grey dashed line, cell means by red dashed line

BEFORE TRANSFORMATION

Plot (3.2) Ion leakage by species, regime, and temperature

−−

−−

−−

−−

−−

−−

−−

−−

−−−−

−−−−

−−−−

−−−−

Degrees

−4

−8

Degrees

Species1

Species2

Species1

Species2

1

2

3

Potato

Ion Leakage

Regime

Cold

RoomTemp

Grand mean depicted by grey dashed line, cell means by red dashed line

AFTER TRANSFORMATION

[Plots (3.1) and (3.2) above depict the equalizing effect on the variance between groups by transforming the response variable. As can be seen by considering the scale of the y-axis, taking the cube root of ion leakage significantly reduced heteroskedasticity between groups resulting in a modified levene test for constant variance p-value of 0.27 (Appendix Table 3.1).]

|  |  |
| --- | --- |
| **Plot (4.1) Potato: Species 1** | **Plot (4.2) Potato: Species 2** |

1.5

2.5

Ion Leakage

−4

Degrees

−8

Degrees

Regime

Cold

RoomTemp

1.5

2.5

−4

Degrees

−8

Degrees

Regime

RoomTemp

Cold

|  |  |
| --- | --- |
| Temp | Temp |
| **Plot (4.3) Regime: Cold** | **Plot (4.4) Regime: Room Temp** |

1.5

2.5

Ion Leakage

Species1

Species2

Temp

−8

Degrees

−4

Degrees

1.5

2.5

Species1

Species2

Temp

−8

Degrees

−4

Degrees

|  |  |
| --- | --- |
| Potato | Potato |

[Plots (4.1-4.4) above depict the two-way interactions, as well as main effects between the different combinations of treatments. Plot

4.2 shows the main effect of the acclimatization regime on ion leakage, Plot 4.4 shows the main effect of potato species on ion leakage, and Plot 4.3 shows the main effect of temperature exposure on ion leakage. Additional profile plots in Appendix Plot 4.]

# Introduction

After observing that plants that have been conditioned to cold weather appear to suffer less damage from cold, researchers designed a 3-factor experiment whereby two different species of potato were acclimatized to either cold or room temperatures, and then later subjected to either -4 degree celsius temperatures or -8 degree celsius temperatures. Two response variables were measured, a damage score for ion leakage and a damage score for photosynthesis. The goal of the study was to determine if, by acclimatizing a plant species to cold weather, the species would be more resilient in cold temperatures.

It is unknown whether the experiment was originally designed to be a balanced design, however, during the experiment some of the plants were lost, and thus the cell sizes were not equivalent between the different levels of treatment. A total of 80 plants were included in the original design, while only 75 plants remained for the duration of the study.

# Methods/Experimental Design

This is a two-cubed design, whereby 3 different factors have 2 levels each. All independent variables are categorical, while the response is continuous. Thus, a three-way ANOVA is appropriate. Due to the unequal cell sizes, a Type III ANOVA is the only appropriate model when considering the full three-way interaction model. A full three-way interaction model will be considered, and model reduction as deemed appropriate will be utilized via manual selection.

The assumptions of this model are as follows:

1. Independence of the error terms (which must be assumed as we cannot test for it due to limited knowledge about the order in which the data were collected).
2. Equal variance between treatment groups, which we will test for using the modified Levene test.
3. Normality of residuals, which we will test for using the Shapiro-Wilk test.
4. No significant outliers, which we will test for using the R-Studentized Residual Test.

All tests, including family confidence coefficients, will be conducted at the *α* = 0*.*05 level and appropriate p-value adjustments will be made when conducting simultaneous hypothesis tests. R will be the sole source of computation.

# Results

A full three-way interaction model was initially fit, and upon diagnostic analysis it was discovered that heteroscedasticity and non-normality of the error terms was present. A modified Levene test centered at the median was conducted and yielded a p-value of < 0.001 (Appendix Table 2.1). This allowed us to reject the null hypothesis of constant variance and was confirmed by the Residuals vs Fitted plot (Plot 1.1). Additionally, non-normality of the error terms was visible in the Q-Q Plot and the Shapiro-Wilk test for normality yielded a p-value of 0.032 (Appendix Table 2.2) allowing us to reject the null hypothesis of normally distributed residuals.

5

15

25

−20

0

20

**Plot (1.1) Residuals vs Fitted**

Residuals

−2

0

1

2

−2

0

2

**Plot (1.2) Normal Q−Q**

Standardized Residuals

# Fitted Values Theoretical Quantiles

[Plot (1.1) shows increasing variance. Plot (2.2) shows non-normally distributed error terms.]

Due to the combination of both non-normality and heteroskedasticity, a Boxcox procedure was implemented to perform transformation of the response variable, which estimated the maximum likelihood estimator of lambda to be *λMLE* = *.*384. Various values of lambda about *λMLE* were implemented, including the powers *Y* 1*/*4, *Y* 1*/*3, and *Y* 1*/*2. Of these 3 simple transformations, cube root was the only transformation that subsequently led to both normally distributed error terms (Appendix Table 3.2) and constant variance (Appendix Table 3.1). It was thus decided that the cube root transformation was the most appropriate, and outliers were then tested for using the R-Studentized Residual test. No outliers were identified (Appendix 3.3).

1.5

2.5

−1.0

0.0

**Plot (2.1) Residuals vs Fitted**

Residuals

−2

0

1

2

−3

−1

1

**Plot (2.2) Normal Q−Q**

Standardized Residuals

# Fitted Values Theoretical Quantiles

[Plot (2.1) shows reasonably constant variance post-transformation. Plot (2.2) shows reasonably normally distributed error terms.]

After transformation, a three-way full interaction model was generated using Type III SS to account for the unequal cell sizes. The three-way interaction term was found to be insignificant (p-value = .4097, Appendix Table 4.1). In order to reduce the complexity, this term was dropped from the model as this F-test was appropriate via the Type III SS method. After fitting the two-way interaction model, diagnostics were again performed which revealed no deviations from assumptions, nor outliers.

Of the two-way interactions, only 1 of 3 was found to be insignificant at the 95% confidence level (Table 5.2), however the decision was made not to reduce the model further as no significant changes were observed by removing the term. Although Potato*∗*Temp was insignificant, both Potato & Temp were involved in the other two interactions. Thus, the main effects would still have to be interpreted at the cell level, so the final type III ANOVA model was as follows:

**Table (5.2) Type III ANOVA Table: Two-way Interaction Only**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Estimate | Std. Error | t value | Pr(*>|*t*|*) |
| (Intercept) | 2.0782 | 0.0599 | 34.67 | 0.0000 |
| Potato1 | -0.3163 | 0.0599 | -5.28 | 0.0000 |
| Regime1 | -0.2792 | 0.0599 | -4.66 | 0.0000 |
| Temp1 | -0.2753 | 0.0556 | -4.95 | 0.0000 |
| Potato1:Regime1 | 0.2884 | 0.0599 | 4.81 | 0.0000 |
| Potato1:Temp1 | 0.0167 | 0.0596 | 0.28 | 0.7800 |
| Regime1:Temp1 | -0.1193 | 0.0595 | -2.01 | 0.0489 |

[Table (5.2): Type III ANOVA showing two significant two-way interaction effects, Potato*∗*Regime and Regime*∗*Temp.]

Because two of the interaction terms were significant, pairwise comparisons using a Tukey family confidence coefficient of 95% were conducted (Appendix Plot (5) & Table (6)) to identify all possible effects, and subsequently identify main effects. 3 main effects were identified, as discussed in the summary, while 13 total contrasts were found to be significant. Of the main effects, only one related to the researchers’ goal as to identify whether acclimatization to cold temperatures increases a plant’s ability to withstand cold weather. Of note, this effect was only significant among species 2 of potato. This effect will be restated here:

“The acclimatization regime significantly affected the mean ion leakage of potato species 2. Specifically, those which were acclimatized to cold temperature showed significantly less ion leakage than those acclimatized to room temperature, regardless of the temperature they were later exposed to.”

The final model coefficients can be obtained via Table (5.1) in the appendix, however it won’t be expressed here as predicting mean ion leakage wasn’t of interest in this experiment – only effects were of interest.

## Conclusion

After transforming the response variable and Using Type III ANOVA SS it was determined that two-way interaction terms were significant. A careful examination of profile plots, combined with Tukey pairwise comparisons at the 95% family confidence level, revealed a total of 13 differences amongst mean response levels. Of these 13, only 3 were considered to be main effects, of which only 1 related to the researchers’ question: Does preconditioning a plant to the cold increase its resistance to cold temperatures?

Species 2 of potato was found to elicit such a response, as the both the profile plots and pairwise comparisons show. Researchers may wish to further study the properties of this species of potato, as it may lead to insight as to a plant’s ability to withstand various temperatures.

Strangely, species 1 of potato exibited significantly more resistance to cold temperatures than species 2 when both species were constrained to room-temperature-acclimatization. This is also cause for further investagation as both species may have advantages when presented with various conditions.