Intra-specific Competition of *Brassica rapa:* Consequences on Plant Health and Development

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Abstract:

In all ecosystems, although it seems never ending, all resources are finite. This scarcity is the definitive catalyst to why competition exists amongst individuals. As a result, there are many circumstances that would drive an organism to contest for needed resources, consequently forcing it to adapt. In our experiment, we replicated intra-specific competition in a contained and controlled area. The experiment was designed to search for statistical differences within or across group relationships in the context of seed density. Our study showed that seed density did have a significant effect on *Brassica rapa's* survivorship, number of flowers, and number of pods. The results confirmed that resource competition between plants of the same species had affected development of an individual and showed potential implications for specialization in the population.

Introduction:

Wherever you look, there is constant competition! On Earth, most species have to compete with their own or against another and much of that is dependent on circumstances not in the hands of the organism. The sphere of competition we have studied is intra-specific competition, a domain where species of the same kind feud for some type of resource in one or many areas.

For example, plants innately lack the physical ability to defend themselves against herbivores, necessitating the development of defenses (Li, Y., *et al*. 2013) or how the location of where a plant is sown impacts its growth priority. Our plant of study was the *Brassica rapa* and in our experiment, we tested the development of flowers or pods, plant height, survivorship, and the total biomass based on seed density. These foundational variables were highly impacted by the "competition-density effect" that we artificially created (Park, S. E., *et al.* 2003, Yoda *et al*., 1963*)*. Other conditions like *B. rapa's* ability to be cultivated into different plants like Turnips, Napa Cabbage, or Bok Choy can lead to certain traits being more emphasized than others. In addition, environmental controls can certainly play a role in development. For example, an adolescent *B. rapa* may be different from another early in the process of becoming a Turnip or a head of Cabbage due to genetics (Shell, J., *et al*. 1993) or because of conditions such as lighting or temperature. (VanDerZanden, A. M, 2022)

The aim of our experiment was to provide an overview on the mechanisms that drive intra-specific competition and the results that come from said competition. The study showed the impact on an individual's growth while also taking into account the implications that come from *B. rapa's* trait specialization and environmental controls. Through the analysis of last year's compiled results and contextual knowledge from our time in Organismal Biology, we hypothesized that the competitive dynamics of intra-specific competition had a direct correlation to overall health and development of an individual and the group as a whole. We also predicted that the density of N seeds sown in a given area would follow a logarithmic, nonlinear curve across all dependent variables.

Methods:

We conducted the experiment by using three green pots with 250 mL of Miracle Grow potting soil. In each pot we first added half of the total soil and then added 6 fertilizer balls. Each pot was then soaked with a couple pipettes of water and the remaining soil was added. Each pot had a different number of seeds in the order 2, 5, or 10. For each seed in their respective pot, a hole was poked and a seed was sown. Each seed was evenly spaced from another, generally clustered towards the inside of the pot. In our class, we had 4 groups of 3 pots and all of the classes' pots were placed into a single clear plastic bin half a foot high. This bin was then slid into a shelf about a foot high and bright white LEDs were used as the light source, with a required distance of 6-8 cm from the source. Lab assistants would then periodically water them. After 5 weeks had passed, we harvested the *B. rapas* and recorded the results.

The extraction and measurement process was done using a shared scale and container. The plants were removed all at once, ensuring the root system was intact. The excess dirt attached to the root system was mostly removed by hand. The number of flowers and pods were noted and each plant length was measured from the base of the stem to the top. The total mass was then recorded by pot.

We used Analysis of Variance (ANOVA) to understand the relationships between seed density to survival rate, biomass, number of flowers and pods, and stem height. Seed density served as our independent variable, while the others were dependent variables. Through this analysis we calculated the F-statistics and the P-values, indicators validating the statistical value of our results. The F-statistics indicated how much each density group (HIGH (10), MED (5), LOW (2)) differed. The P-value determined if the found differences were statistically significant. Post-Hoc analysis was performed on all categories, with Meandiff, P-adj, Lower and Upper as indicators to determine significant differences between seed density pairs in their respective groups. Tukey's Honestly Significant Differ was used to find the mean values amongst a group of means which determined if density pairs were different. Meandiff represents the difference between treatment groups, which is bounded by the Lower and Upper values. The Lower and Upper results define the range most likely to include the true mean difference. P-adj represents statistical significance, just like the P-value, however it is adjusted to compensate for false rejections of true null-hypotheses. This adjustment is implemented through Studentized Range Distribution, a method used to measure how exaggerated a range of means is relative to what is expected by chance. ANOVA and Post-Hoc calculations were performed using Juypter Notebook and Python, implementing the libraries Pandas, Numpy, Scipy's ANOVA method, Statsmodels pairwise_tukeyhsd method, and Matplotlib for post-hoc visualization . In order to calculate the data set, values 'NaN' or '#DIV/0!' were replaced with 0s and were included into the calculations. Box-plots and bar graphs were created using Data Explorer, an Azure database implemented with Kusto Query Language and Python, following similar handling of 'NaN' and '#DIV/0!' values. (Shsagir., *et al.* 2023)

Results:

Before P-values and F-statistics were calculated, non-applicable cells were all replaced with 0 (Figure 1). Survivorship Wk5, Average # Flowers, and Average # Pods all indicated a significant difference in mean numbers of associated categories, across all density treatments (ANOVA: Survivorship Wk5: F = 4.345 P = 0.0136, Average # Flowers: F = 20.554 P = 3.028^{-9}, Average # Pods: F = 20.073 P = 4.696^{-9}). Average Height (mm) and Total Biomass both did not indicate a significant difference in mean numbers for all density treatments (ANOVA - Average Height (mm): F = 1.122 P = 0.327, Total Biomass = F = 1.694 P = 0.186) (Figure 2). The Average # Flowers and Average # Pods showed the strongest indication of statistical value, while Survivorship Wk5 did show differences but did not indicate that the variation was as strong as the number of flowers and pods. In contrast, Average Height (mm) and Total Biomass did not show much variation amongst groups and the P-values supported this conclusion. Post-hoc analysis was performed on all dependent variables with an alpha of 0.05, following the comparison order of HIGH-LOW (H_L), HIGH-MED (H_M), LOW-MED (L_M). Average Height (mm) (P-ADJ - H_L = 0.2983, H_M = 0.6392, L_M = 0.8286) and Total Biomass (P-ADJ - H_L = 0.1624, H_M = 0.5068, L_M = 0.7557) were also concluded to be insignficant.

In the case of Average # Pods (P-ADJ - H_L = 0.0, H_M = 0.0013, L_M = 0.0158) and Average # Flowers (P-ADJ - H_L = 0.0, H_M = 0.0403, L_M = 0.0003) both rejected the null hypothesis, indicating that the differences between treatments were indeed significant. Survivorship WK5 indicators (P-ADJ - H_L = 0.0095, H_M = 0.2713, L_M = 0.3406) showed significant differences between high and low densities but did not for high to medium and low to medium densities.(Figure 3)

Discussion:

The hypothesis made by the group was supported and concluded that there was a direct correlation between the density of seeds sown in a given area with the development of an individual and the whole population. Observations on flower and pod development are considered the most significant, with both ANOVA and Post-Hoc calculations strongly suggesting a relationship with the given treatment. In contrast, the total biomass and average height of the data set was considered insignificant by both methods but the total biomass correlation to density was noticeably stronger than the average height. A reason for this may be because of the close and direct light source. This is related to the lack of overcast that typically occurs in non-agronomic conditions. In addition, the containers for the plants lacked depth and were routinely watered, discouraging deep root systems, perhaps affecting the overall biomass. More so, the lateral distances between plants was much tighter than the availability of vertical space and could suggest that vertical space plays a large role in biomass. The particularly interesting data point lies in the survivorship's relationship with density. ANOVA metrics for this group indicated correlation but upon closer inspection, in the Post-Hoc analysis, paired treatment data showed very strong statistical significance between high and low densities. Given that the paired treatment comparison were the extremities of the survivorship data, it poses an interesting suggestion in light of our groups given prediction. Although our prediction was wrong for the other categories, the survivorship group implies a ‘ceiling’ on the population capacity of a given space.This supports the idea that if the total population’s needs surpasses the environment's available resources, then the total mass equals those resources. (Figure 4)

Indicators of Lower and Upper across all dependent variables for pair groups L_M suggest that this treatment pair is the least significant in value, despite some groups rejecting the null (Figure 3). Additional data points that should be taken into consideration can be found in Figure 1, where the total number of invalid inputs are shown. Improvements in the experiment can be done by recording the length of the root system and also measuring the mass of the roots separate from the stem. This can be used to find genetic predispositions when the controls of the experiment are adjusted, such as vertical space or water availability. Additionally, complete removal of excess dirt will improve the measurements in regards to total biomass. In conclusion, the experiment showed that seed density plays a large role in bud and flower development perhaps due to the availability of nutrients while height and total biomass are more affected by other variables such as the availability of vertical space or time spent in the light. Survivorship seemed to fit in the middle of the other four categories suggesting that there are more factors that play into the results of plant survivorship.

Figures:

Figure 1:

```
'High: '

# Seeds Planted        0
Survivorship Wk5       0
Average # Flowers      2
Average Height (mm)    4
Average # Pods         3
Total Biomass          7
dtype: int64

'Med: '

# Seeds Planted        0
Survivorship Wk5       0
Average # Flowers      3
Average Height (mm)    3
Average # Pods         5
Total Biomass          7
dtype: int64

'Low: '

# Seeds Planted        0
Survivorship Wk5       0
Average # Flowers      7
Average Height (mm)    3
Average # Pods         9
Total Biomass          7
dtype: int64
```

Figure 2:

```
Survivorship Wk5: F-Value: 4.344637254522216, P-Value: 0.013560213015472153
Average # Flowers: F-Value: 20.55386109857788, P-Value: 3.0276985842356508e-09
Average Height (mm): F-Value: 1.1219340653581618, P-Value: 0.3266164240227396
Average # Pods: F-Value: 20.072799642339046, P-Value: 4.695994752707304e-09
Total Biomass: F-Value: 1.6935666818621093, P-Value: 0.18510683706014305
```

Figure 3:

'Survivorship Wk5'

Multiple Comparison of Means - Tukey HSD, FWER=0.05

group1	group2	meandiff	p-adj	lower	upper	reject
high	low	11.6901	0.0095	2.3591	21.0212	True
high	med	6.1268	0.2713	-3.2043	15.4578	False
low	med	-5.5634	0.3406	-14.8945	3.7677	False

'Average # Flowers'

Multiple Comparison of Means - Tukey HSD, FWER=0.05

group1	group2	meandiff	p-adj	lower	upper	reject
high	low	4.6356	0.0	2.9198	6.3515	True
high	med	1.7774	0.0403	0.0616	3.4933	True
low	med	-2.8582	0.0003	-4.5741	-1.1424	True

'Average Height (mm)'

Multiple Comparison of Means - Tukey HSD, FWER=0.05

group1	group2	meandiff	p-adj	lower	upper	reject
high	low	46.2594	0.2983	-26.9256	119.4443	False
high	med	28.0756	0.6392	-45.1093	101.2605	False
low	med	-18.1838	0.8286	-91.3687	55.0011	False

'Average # Pods'

Multiple Comparison of Means - Tukey HSD, FWER=0.05

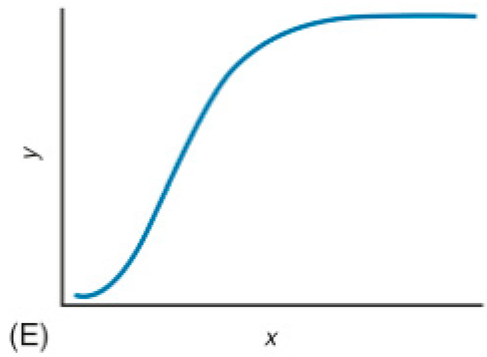
group1	group2	meandiff	p-adj	lower	upper	reject
high	low	6.0753	0.0	3.8145	8.336	True
high	med	3.4073	0.0013	1.1466	5.6681	True
low	med	-2.668	0.0158	-4.9287	-0.4072	True

'Total Biomass'

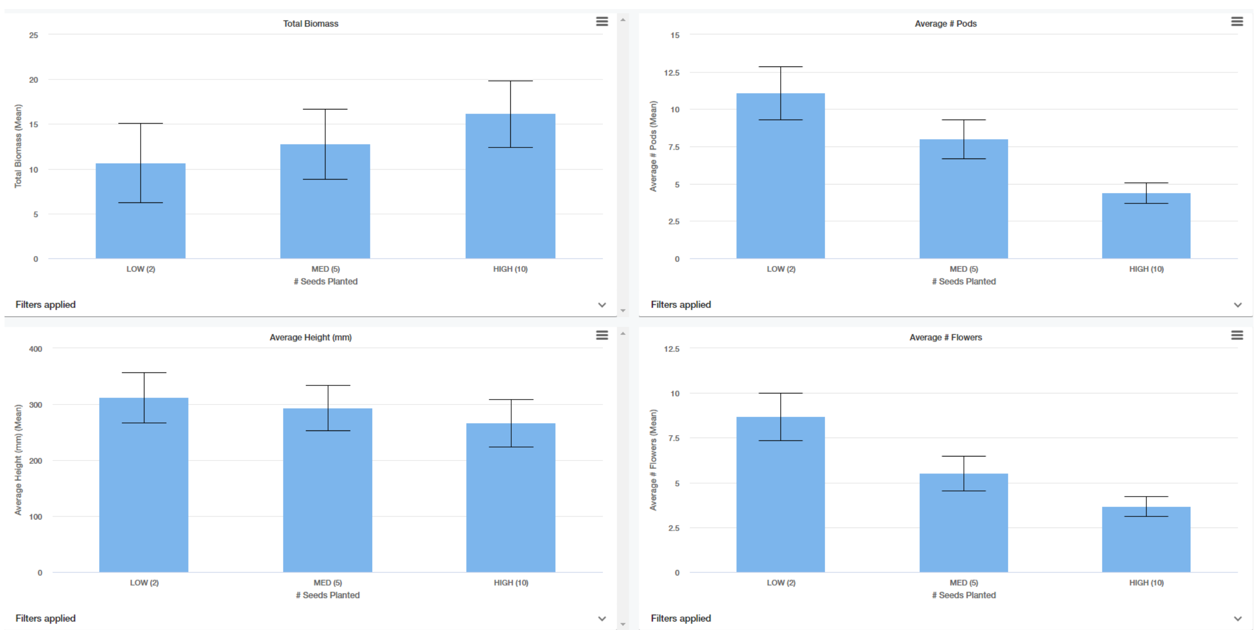
Multiple Comparison of Means - Tukey HSD, FWER=0.05

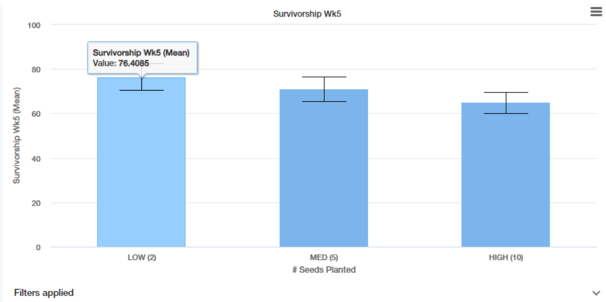
group1	group2	meandiff	p-adj	lower	upper	reject
high	low	-5.2144	0.1624	-11.931	1.5023	False
high	med	-3.1772	0.5068	-9.8939	3.5395	False
low	med	2.0372	0.7557	-4.6795	8.7538	False

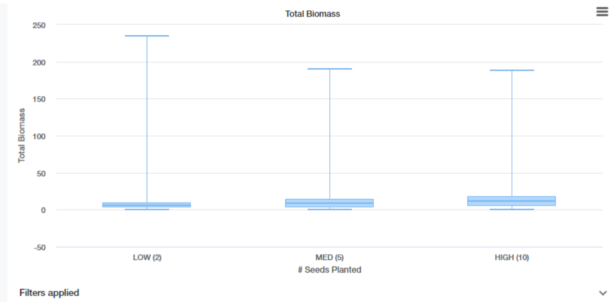
Figure 4:

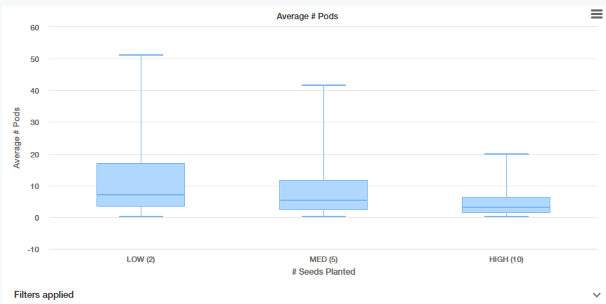


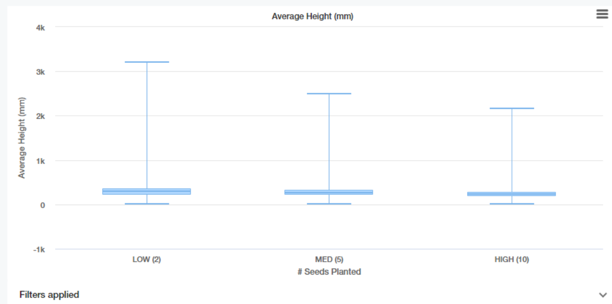
Figures 5 (ANOVA):

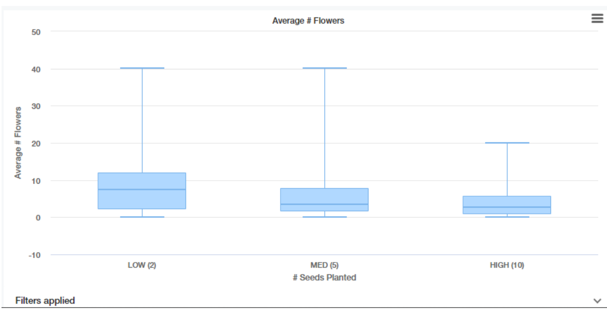


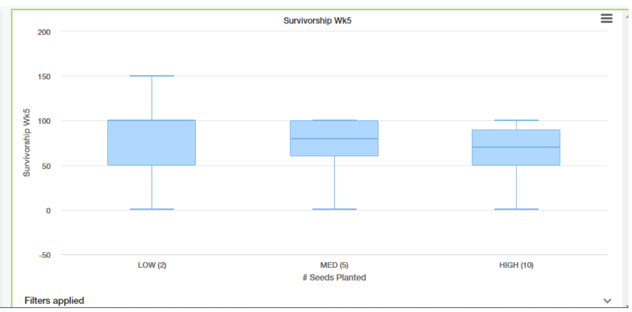

Survivorship Wk5
Survivorship Wk5 (Mean)
Survivorship Wk5 (Mean)
Value: 76.4085
100
80
60
40
20
0
LOW (2)
MED (5)
HIGH (10)
Seeds Planted
Filters applied


Total Biomass
Total Biomass
250
200
150
100
50
0
-50
LOW (2)
MED (5)
HIGH (10)
Seeds Planted
Filters applied

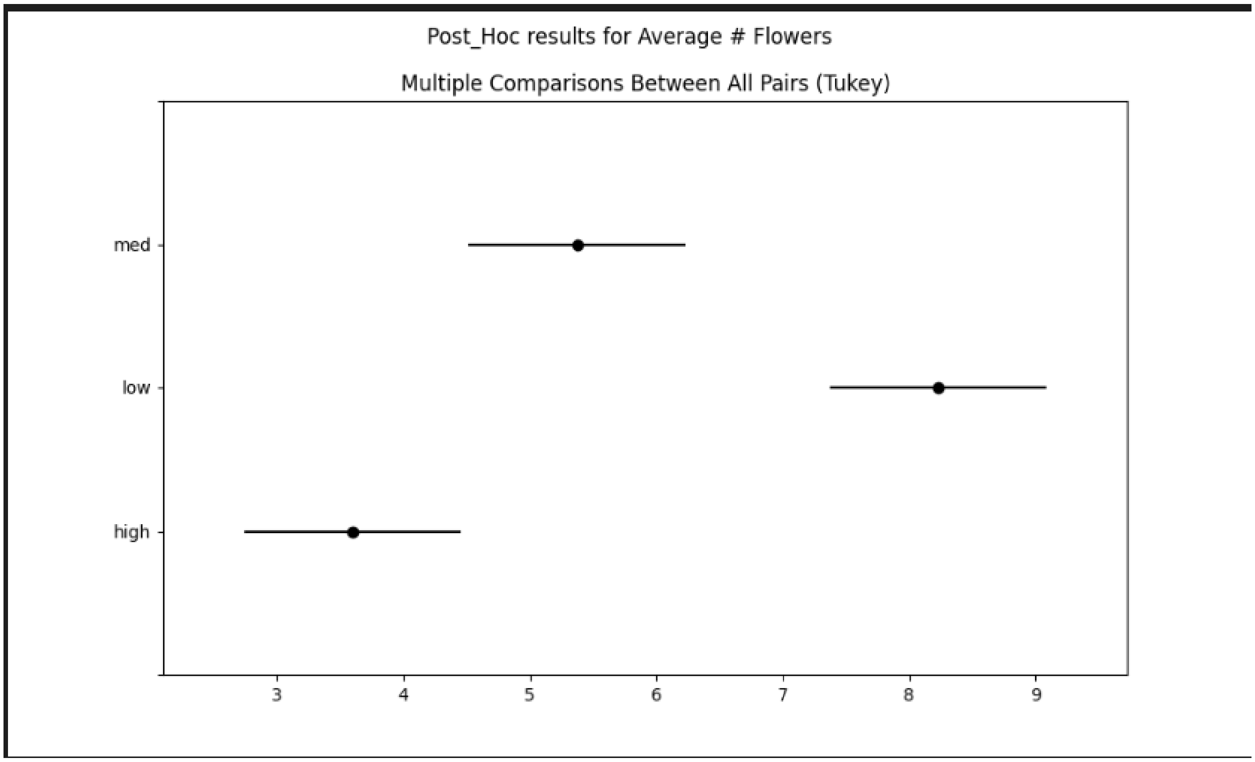

Average # Pods
Average # Pods
60
50
40
30
20
10
0
-10
LOW (2)
MED (5)
HIGH (10)
Seeds Planted
Filters applied


Average Height (mm)
Average Height (mm)
4k
3k
2k
1k
0
-1k
LOW (2)
MED (5)
HIGH (10)
Seeds Planted
Filters applied


Average # Flowers
Average # Flowers
50
40
30
20
10
0
-10
LOW (2)
MED (5)
HIGH (10)
Seeds Planted
Filters applied

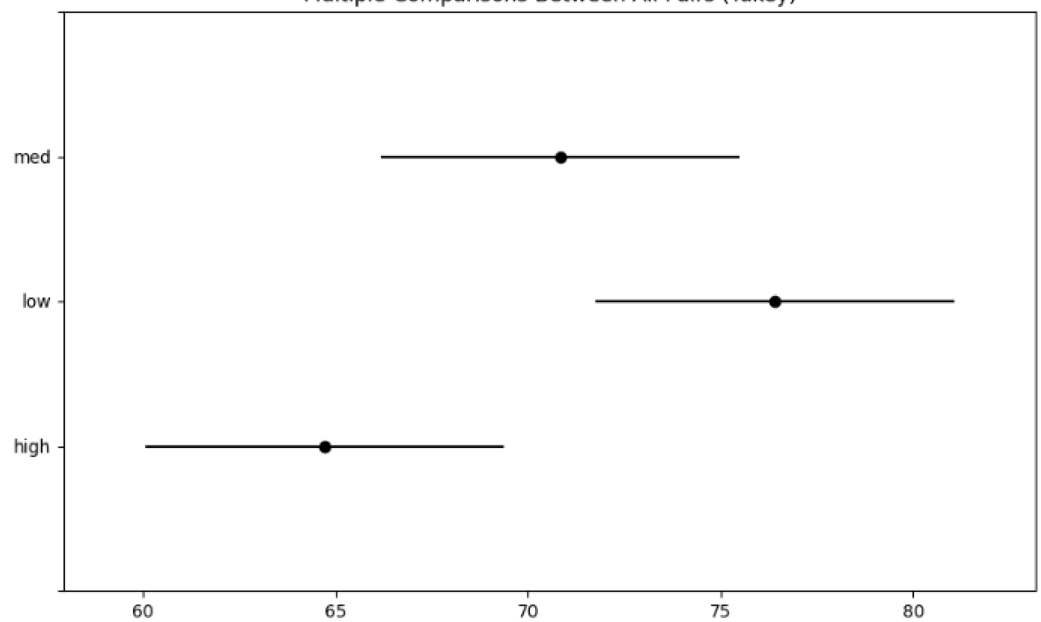

Survivorship Wk5
Survivorship Wk5
200
150
100
50
0
-50
LOW (2)
MED (5)
HIGH (10)
Seeds Planted
Filters applied

Figures 6 (POST-HOC):



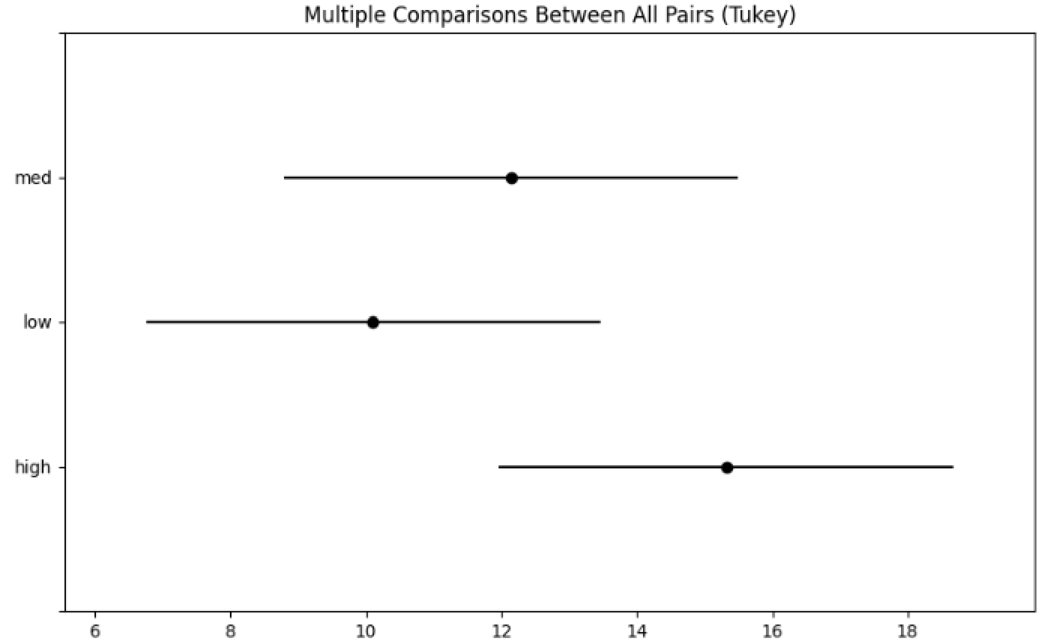
Post_Hoc results for Survivorship Wk5

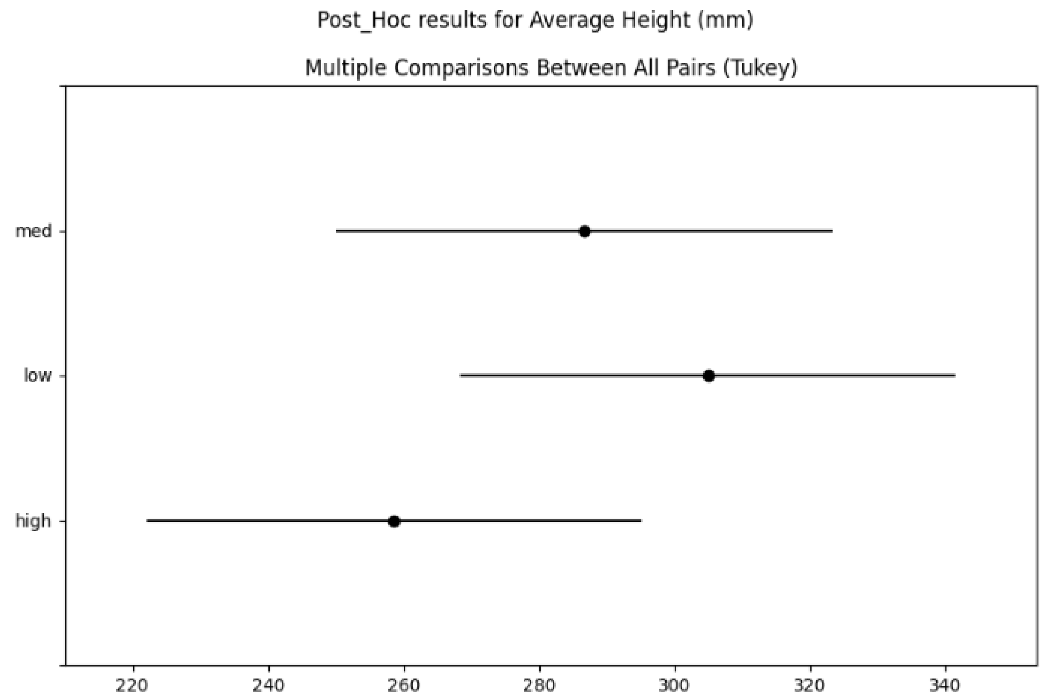
Multiple Comparisons Between All Pairs (Tukey)

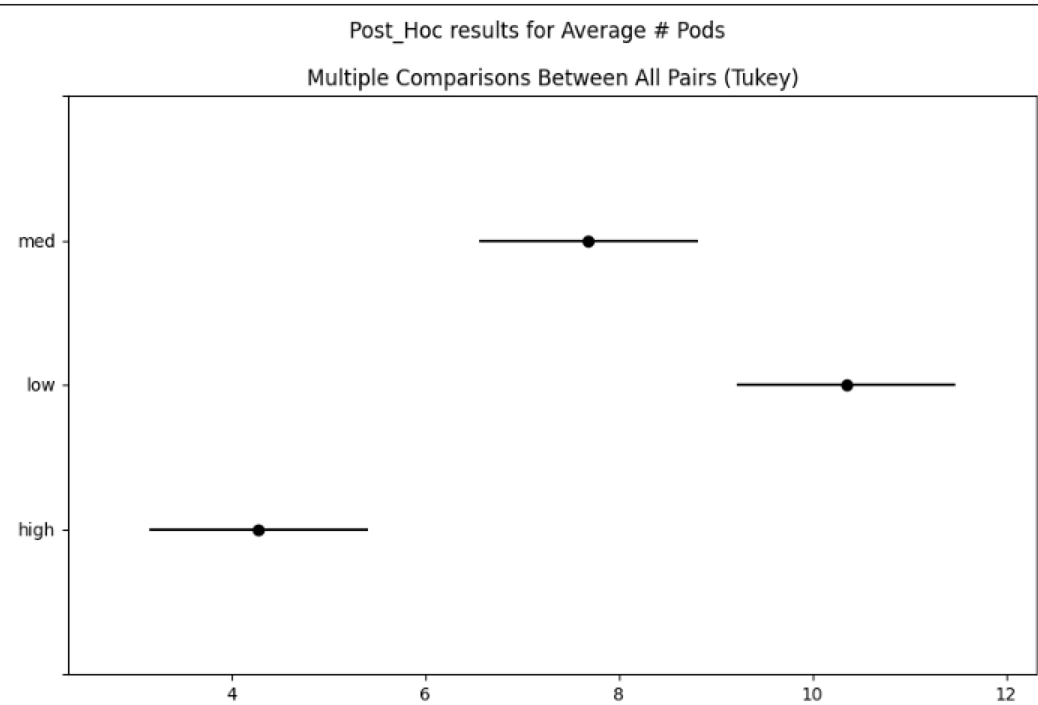


Post_Hoc results for Total Biomass

Multiple Comparisons Between All Pairs (Tukey)




Post_Hoc results for Average Height (mm)
Multiple Comparisons Between All Pairs (Tukey)
med
low
high
220
240
260
280
300
320
340


Post_Hoc results for Average # Pods
Multiple Comparisons Between All Pairs (Tukey)
med
low
high
4
6
8
10
12

References:

Li, Y., et al. (2013). Intra-specific variation in wild Brassica oleracea for aphid-induced plant responses and consequences for caterpillar–parasitoid interactions. *Oecologia*, *1* (2), 1. retrieved from https://doi.org/10.1007/s00442-013-2805-6

Park, S. E., et al. (2003, December). The theory and application of plant competition models: An agronomic perspective. *PubMed Central*. U.S. National Library of Medicine. (3) retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4243621/

Shell, J., et al. (1993, December 15). Genes involved in the control of growth and differentiation in plants. *PubMed Central*. U.S. National Library of Medicine. *135* (3) 245-249 retrieved from https://pubmed.ncbi.nlm.nih.gov/8276264/

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YodaK, Kira T, Ogawa H, Hozumi K.1963**.** Self‐thinning in overcrowded pure stands under cultivated and natural conditions (intraspecific competition among higher plants XI). *14* (3): 107–129 retrieved from *Journal of Biology, Osaka City University*. [Google Scholar]

Shsagir., et al. (2023, October 13). Ingest data using the Azure Data Explorer Python library. Azure Data Explorer | Microsoft Learn. Retrieved from https://learn.microsoft.com/en-us/azure/data-explorer/python-ingest-data.

Source Code: https://github.com/Cutupnottoasted/Brassica-rapa-Analysis