Effects Of Nitrogen And Potassium-Based Fertilizers On Green Peach Aphid and Abundance And Arugula Condition

Rith Bal, Megan Groshok & Yahya Jama

Integrated Science Program, Class of 2024, McMaster University

SUMMARY

Both soil nutrient uptake and pest infection have a significant impact on agricultural plants. Through the use of arugula (Eruca vesicaria) and the green peach aphid (Myzus persicae), this study aims to better understand the effects that human manipulation has on the growth and condition of a plant, as well as the effects of fertilizers on aphids. Arugula plants were treated with recommended and high levels of nitrogen and potassium-based fertilizers as well as the absence or presence of green peach aphids. Aphid count, leaf count and arugula condition were observed. Nitrogen fertilizers resulted in an increase in aphid count from the control while potassium fertilizers caused a decrease in aphid count. Arugula leaf count was also observed to have a positive correlation to aphid count, with a medium leaf count of eight producing a significantly higher aphid count than any leaf count. Furthermore, nitrogen fertilizer was found to improve arugula condition, the effect increasing with the concentration of nitrogen, although there was not a significant effect on arugula growth. Finally, the recommended dose of potassium benefits arugula growth, while the high dose harms arugula growth. Potassium was found to not have a significant effect on arugula condition. This study hopes to inform the agricultural community of the impacts fertilizer has on crop production and the greater ecological world.

Received: 11/15/2021 **Accepted:** 03/08/2022 **Published:** 03/03/2024

Keywords: Green peach aphids, arugula, fertilizer, nitrogen fertilizer, potassium fertilizer, plant-animal interactions, population dynamics, plant condition

INTRODUCTION

It is imperative to understand the effects of anthropogenic exploitations on the ecosystem. Due to the increased demand for agricultural products, many farmers have turned to fertilizers to increase crop productivity and meet the rising demand of the public (Elferink and Schierhorn, 2016). Two of the most commonly used fertilizers are nitrogen and potassium-based fertilizers due to their importance in plant growth, defence, and structure (Kissel and Harris, n.d.). Another issue agriculturalists must contend with is pest control. Pests have a considerable impact on agricultural cultivation and are further explored in this study. The system investigated in this study is the effects of the herbivorous pest green peach aphids on the arugula plant. Although many people have studied the effects of fertilizers on plants, there is markedly less research investigating the effect of fertilizers on herbivores.

In any ecosystem, numerous factors must be kept in balance for the ecosystem to function optimally—otherwise, the ecosystem can suffer and potentially collapse. These factors vary widely from one ecosystem to another and may include resource availability, climate, and interspecific interactions (Cleland, 2011). One such example is plant-animal interactions, specifically between herbivore and plant populations. A critical determinant of the success of herbivore-plant interactions includes the resources available to plants. For example, the nutrients a plant uptakes through soil have a variety of effects on its growth (Cleland, 2011). Understanding the effects that these soil nutrients have on plants, particularly when they are manipulated by humans, is crucial to the understanding of their effects on other parts of the ecosystem, including herbivores.

In existing literature, there are few studies that investigate the effects of fertilizers and soil composition on green peach aphids. The research revealed two key studies in this area, although neither study included arugula. The first, by Grechi et al. (2010), investigated the effects of nitrogen fertilizer on aphids, using the study system of Prunus persica (peach tree). Researchers found that nitrogen had a positive effect on aphid population size at intermediate doses and a negative effect on aphid population size at high doses (Grechi et al., 2010). Another key finding was that plant growth only partially correlated with aphid population dynamics, and that changes in plant chemistry — including plant defence-related metabolites and amino acids — did not account for this difference (Grechi et al., 2010). The other study, performed by Sarwar, Ahmad, and Tofique (2011) investigated the impact of soil potassium on aphid population size and crop yield using Brassica napus (canola). Like arugula, canola is in the Brassicaeae family. The findings showed that aphids flourished in plants grown without potassium (Sarwar, Ahmad, and Tofique, 2011). Additionally, Sarwar, Ahmad, and Tofique (2011) found 100 kg and 120 kg applications of potassium per hectare produced the least aphids per plant with the highest seed yield (Sarwar, Ahmad, and Tofique, 2011). This study aims to expand this body of research through a different study system, arugula, while utilizing nitrogen and potassium-based fertilizers to directly compare their effects. This study utilizes varying levels of nitrogen and potassium to determine their effects at different dosages.

STUDY SYSTEM

Arugula is an annual herbaceous plant part of the Brassicaceae family (Silva et al., 2021). It is native to the Mediterranean and Near East but is grown across the world for human consumption in both fertile and poor soil (Tripodi et al., 2017). Arugula is about 15 cm tall on average with broad leaves that have a strong flavour. Furthermore, the plant's constitutive defence consists of trichomes as well as toxic glucosinolates and anti-nutritive protease inhibitors as an induced defence mechanism (Ogran et al., 2020). Members of the Brassicaceae family make up a great deal of agriculturally and commercially important products including broccoli, cabbage, and canola (Macdonald, 2017). As such, the information on the reactions of arugula to fertilizers and aphids gained from this study can provide valuable insight into the reactions of similar plants.

Arugula also acts as an essential nutrient source for many predators, including green peach aphids (Little et al., 2011). Native to Europe (Long and Riedl, 1993), green peach aphids have spread across the world and are the herbivore of interest in this study. The species is characterized by its yellow-green colour and is about 2mm in size. This species feeds on plant phloem, stunting plant growth and suppressing photosynthesis (Sudderth and Sudderth, 2014).

Moreover, aphids feed on hundreds of plants from over 40 different plant families, a great deal of which are agricultural crops (Capinera, 2001). As such, green peach aphids are an impactful species in agriculture, and it is incredibly beneficial to explore the factors that affect aphid population growth. It is also noteworthy that the use of nitrogen fertilization as a method of pest management is a common cropping practice (Capinera, 2001). Therefore, thiaim to understand the effect that manipulating soil nutrient levels using nitrogen and potassium-based fertilizers has on green peach aphid populations, as well as the growth and condition of their host plants.

RESEARCH QUESTION AND HYPOTHESES

This investigation attempts to answer two foundational research questions. The first question asks: how do nitrogen and potassium-based fertilizers affect green peach aphid population growth dynamics? The secondary analysis investigates how arugula condition and growth affect green peach aphid abundance. The second question asks: how do nitrogen and potassium-based fertilizers affect arugula growth? The study hypothesizes that the most optimal treatment for aphid population regulation will be the higher dose of the 0-0-60 (Nitrogen-phosphorus-potassium percentages) potassium fertilizer, as an abundance of potassium in soil has been proven to decrease aphid density (Sarwar, Ahmad, and Tofique, 2011) and higher nitrogen levels have been linked to increased aphid survival and fecundity (Grechi et al., 2010). It is also hypothesized that the optimal treatment for arugula condition will be the recommended dose of the 34-0-0 nitrogen fertilizer. Nitrogen is the second most absorbed nutrient identified in arugula leaf tissue and is a fundamental component of leafy plant development and growth (Liu et al., 2014; Grangeiro et al., 2011). Consequently, inadequate levels of nitrogen have been associated with lower photosynthetic activity and reduced leaf growth (Grangeiro et al., 2011). Potassium is a nutrient critical for arugula root and stem growth but is considered the third most important nutrient in commercial fertilizer (Xu et al., 2020). Overuse of potassium has been linked to inhibited root development (Sarwar, Ahmad, and Tofique, 2011). Ultimately, it stands to reason that the recommended dose of nitrogen will be the optimal treatment for arugula condition.

MATERIALS AND METHODS

Study Site

This study was conducted for 13 days between Thursday, September 23 2021 and Tuesday, October 5, 2021 at the Integrated Science lab at McMaster University in Hamilton, Canada. The trays holding each of the arugula plants were placed on a counter behind large windows facing north. The plants were exposed to sunlight for 11.5 to 12 hours each day and although the windows were not covered by blinds, they were at times shaded by a large tree outside the window, so the plants did not receive constant direct sunlight throughout the day.

Experimental Design

This investigation explores the effects of varying fertilizer contents and concentrations on aphid population growth dynamics and overall arugula growth. By using recommended and high doses of potassium (84 kg/hectare and 178 kg/hectare, respectively) and nitrogen (95 kg/hectare and 140 kg/hectare, respectively) fertilizers (Oregon State University, 2010; Kissel and Harris, n.d.), this study intends to observe aphid population growth to determine the ideal treatment conditions for arugula condition as well as pest control. Therefore, this lab contrasts the concentrations of potassium or nitrogen against green peach aphid infection, leaf count, and arugula condition of each treatment at the conclusion of the experiment. This experiment utilizes a two by three by two factorial design. In sum, ten treatment groups are used, including two treatments of green peach aphids (not present or present), three soil treatments (nitrogen, potassium, control), and two levels of nitrogen and potassium (recommended and high doses). Each treatment was repeated six times for a total of 60 samples. Each sample consisted of one arugula plant and one out of the 12 potential combinations of treatments including two controls. The controls, or untreated samples were the same, as there were no added nutrients. These groups were combined, resulting in a total of ten treatment groups.

To prepare the treatment solution, two concentrations of potassium (0.1506 grams and 0.7562 grams, respectively) and two concentrations of nitrogen (0.1329 grams and 0.3655 grams, respectively) were measured using a digital scale. Each of these concentrations were then dissolved in a 1 L beaker of distilled water using a magnetic stirrer to create four 1 L stock solutions. Next 50 mL of stock solution was poured into a 100 mL graduated cylinder and was applied to all the plants in each of the treatment groups (recommended nitrogen, recommended potassium, high nitrogen, high potassium), excluding the control group. Each of the plants in the control groups received 50 mL of distilled water.

In total, four trays were used. Trays one and two contained samples with aphids while trays three and four contained samples without aphids. Five aphids were applied to each individual arugula plant in trays one and two on day 0.

Tray one contained both the recommended and high doses of nitrogen for a total of 12 samples. Tray two contained the recommended and high doses of potassium, as well as a control for a total of 18 samples. Tray three contained the recommended and high doses of nitrogen for a total of 12 samples. Tray four contained the recommended and high doses of potassium, as well as the other control for a total of 18 samples. When left to grow, the order and orientation towards the window of each tray were randomized to ensure minimal bias. This experimental setup is depicted in Figure 1.

Twelve random arugula plants were assigned to each of the five treatments. From the total of 60 arugula plants, six plants from each treatment group (recommended nitrogen, recommended potassium, high nitrogen, high potassium, or control) were inoculated with aphids, for a total of 30 samples. Each one of these 30 plants were inoculated with five green peach aphids on day 0. These plants inoculated with aphids, were placed in a covered tray separate from the ones without aphids.

Climatic Conditions

The room temperature was at ambient room temperature 25 ℃ (298.15K). The assumption is that the temperature trays did not differ significantly from the outside environment.

Plant Seeding and Insect Rearing

The Roquette arugula seeds were sown on August 10, 2021. The soil contained 0.30% total nitrogen, 0.12% available phosphoric

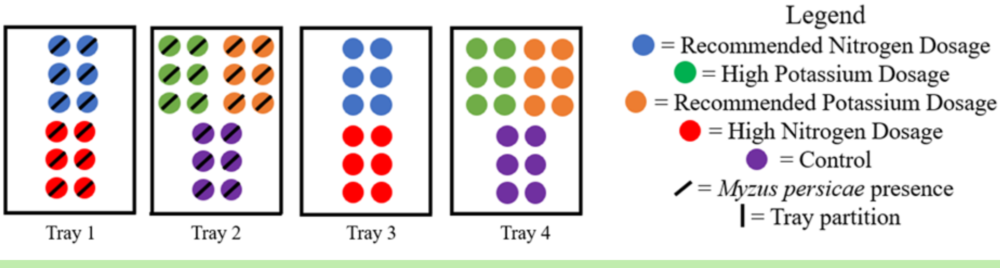

Figure 1: Graphical depiction of the experimental set-up. From left to right, the trays are ordered tray one, tray two, tray three, and tray four. When left to grow, the order and orientation of the trays were both randomized after each time that data was collected. Each tray had identical dimensions. All five treatments are depicted in this figure, and it is clear that plants inoculated with aphids and plants without aphids were segregated.

acid, 0.10% soluble potash, 0.17% calcium, 40% organic matter, and 65% maximum moisture. The soil was watered and given 12 hours of sunlight per day. The aphid nymphs were raised on tobacco leaves prior to the lab.

Bioassay and Data Collection

Data collection began on Friday, September 24, which was 46 days after seeding and one day after the experimental setup. The data that was collected included each plant's aphid count, mesophyll leaf count to determine plant growth, and each plant's condition was graded according to the Suddaby Level (SL) (Suddaby et al., 2008). The SL was used to classify each plant's condition on a scale from one to four, where zero indicates that the plant was dead and four indicates that the plant is in optimal condition. On data collection days, each individual plant was observed, and then the SL scale was used to determine which rating the plant fell into according to its symptoms. Data collection was performed on six days over a period of 13 days starting on September 24 as day 1, September 27 as day 2, September 29 as day 3, October 1 as day 4, October 4 as day 5, and October 5 as day 6.

Additional observational data was collected on new leaf emergence by marking new leaves with a black non-toxic permanent marker. The watering schedule for each plant was done as needed on the dry plants based on the Texas A&M Agrilife method (Texas A&M Agrilife Research and Extension Center, 2021). This observational data was collected to provide additional data on plant growth and aphid population distribution.

STATISTICAL ANALYSIS

For the statistical analysis of the data obtained in this study, one, two, and three-way analyses of variance (ANOVA) were performed using linear models in R (R Core Team, 2021). These facilitated the examination of the effects of each independent variable—the absence or presence of aphids along with the fertilizer treatment—on aphid count, leaf count and arugula condition, which are the dependent variables. An additional ANOVA was performed to investigate the effects of the dependent variables on each other, as the study aims not only to determine the effect of fertilizer on each variable, but also how aphids affect and are affected by the condition and growth of arugula. To determine if an interaction showed a significant effect, the adjusted R-squared value, P-value, and F-statistic were analysed. If an interaction showed a significant relationship in an ANOVA, a post-hoc Estimated Marginal Means test was performed, to determine exactly which relationships within the data were significant through the multiple pairwise comparisons. The estimated marginal means post-hoc test was chosen because it best facilitates the study of interactions, and it clearly displays significant interactions.

RESULTS

Aphid Population Performance

After conducting a two-way ANOVA, it was observed that there was no significant effect of treatment and time on aphid count. There was, however, a significant effect of treatment on aphid count, seen in Table 1. For plants inoculated with aphids, the highest mean aphid population at the conclusion of the experiment (day 6), were the samples treated with the recommended dose of nitrogen fertilizer (Figure 2). This indicates that aphid populations fare best with the recommended nitrogen fertilizer treatment. Contrarily, the recommended dose of potassium corresponded to the smallest mean aphid population. There was no significant difference between the mean aphid count of the recommended and high potassium treatments. Furthermore, the two nitrogen treatments, recommended and high, were observed to produce the highest mean aphid counts, while the recommended and high potassium treatments produce the two smallest aphid counts. There was a significant difference between the mean aphid counts of the recommended treatment of nitrogen and the recommended and high treatments of potassium fertilizers. Out of all five treatments, the untreated soil (control) had

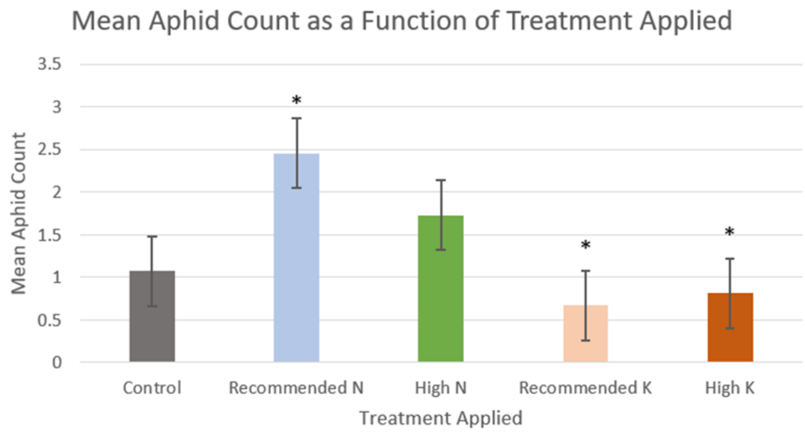

Figure 2: Graphical representation of the mean aphid count (± 0.408SE) as a function of the five treatments: control, high or recommended nitrogen, and high or recommended potassium. A significant difference in mean aphid count can be seen between the recommended dose of nitrogen and the high and recommended doses of potassium. To construct this plot, a two-way ANOVA examining the effects of treatment and time was completed. The combined effect of treatment and time on aphid count was insignificant ($F_{24,385} = 0.476$, $P = 0.984$), but the effect of treatment on aphid count was statistically significant ($F_{4,385} = 3.23$, $P = 0.01265$). An Estimated Marginal Means test was then performed to determine the significantly different (*) treatments in this study.

the intermediate mean aphid count. In totality, it was observed that the two nitrogen treatments produce the highest mean aphid count, the control produces the intermediate mean aphid count, and the two potassium treatments produce the lowest mean aphid counts.

Effects of Leaf Count and Plant Condition on Aphid Population

As a secondary analysis on the factors that affect aphid count, an analysis was also performed on the effects of plant condition and leaf count on aphid count. Ultimately, it was determined that plant condition and leaf count did not have a significant combined effect on aphid count. The corresponding p-values and F-values, as well as those for subsequent comparisons are shown in Table 1. However, there is a significant effect of leaf count on aphid count. Subsequent analysis considering both leaf count and the initial absence or presence of aphids (Figure 3) revealed an even stronger relationship between leaf count and aphid count, as the analysis focused on only the plants exposed to aphids. Overall, it was determined that a leaf count of eight resulted in a significant difference in aphid count compared to plants with any other leaf count. It was confirmed that this was not due to any singular outlier plants. Throughout the course of the experiment, numerous plants showing notably high aphid counts were observed in three different treatment groups: recommended nitrogen, high nitrogen, and control. This is consistent with the primary findings that the recommended nitrogen, high nitrogen and control treatment groups experienced higher mean aphid counts. Thus, the results suggest that a leaf count of eight is optimal for a maximal aphid count.

Fertilizer Presence on Arugula Condition

WITHOUT APHIDS

The addition of the high nitrogen treatment to arugula plants without aphids resulted in the plants having a mean SL of 3.8 (Figure 4). This is significantly higher and in contrast with the control plants that had a mean SL of 3.0. Moreover, the high nitrogen treatment group had a significantly higher mean SL of 3.8 relative

Table 1: Summary table detailing the relevant statistical values for the one, two, and three-way analyses of variance performed. These values were calculated based on the data collected over the 13-day period of data collection. The dependent variables used are Suddaby level, aphid count, and leaf count. The independent variables used are Suddaby Level, fertilizer treatment, aphid presence, day, and leaf count.

Dependent Variable	Effect	d.f.	Pr(>F)	F-value
Aphid count	Treatment:Aphids:Day	4	0.0127	3.23
Aphid count	Suddaby	4	0.595	0.696
Aphid count	Suddaby:Leaf count	25	5.67e-07	2.05
Aphid count	Leaf count	11	1.33e-11	7.40
Leaf count	Treatment:Aphids	4	4.96e-09	11.87
Suddaby	Treatment:Aphids:Day	24	1.09e-05	7.35

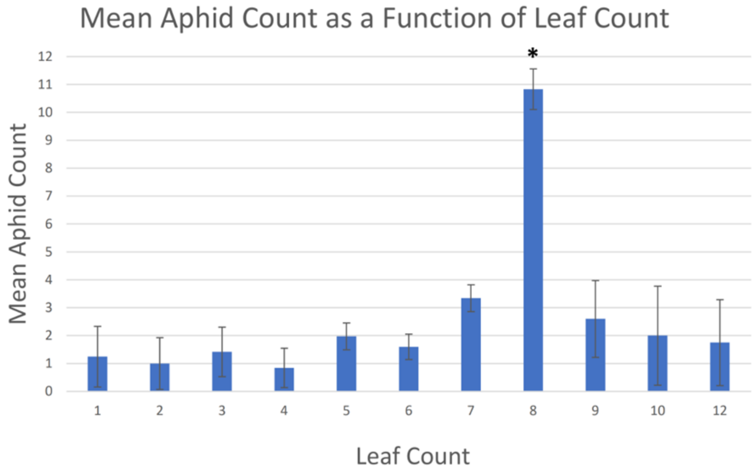

Figure 3: The effect of leaf count on the mean aphid count of an arugula plant. No plants with 11 leaves were observed over the course of the experiment and dead plants were excluded from the graph as aphids do not reside on dead plants. This data only includes the plants belonging to groups that were initially inoculated with aphids. An Estimated Marginal Means post-hoc test considering the effect of leaf count and the presence of aphids on aphid count showed that plants with eight leaves showed a significantly higher mean aphid count ($F_{40,379} = 2.694$, $P = 5.67e-07$) than plants with any other leaf count.

to the recommended nitrogen group which has an average SL of 2.9. This result suggests that high nitrogen-rich soils support the improvement of arugula condition more than unfertilized soil and recommended nitrogen soil. There were no other significant differences in arugula condition in the high and recommended potassium groups.

WITH APHIDS
Among the arugula plants infected, the recommended potassium treatment group had the highest mean SL of 3.6, which was significantly higher than the infected high nitrogen group's mean SL of 2.9. There were no significant differences between the high nitrogen, recommended nitrogen group, or control.

WITH AND WITHOUT APHIDS
In the analysis of the plants infected with aphids, the high nitrogen group was found to have the lowest mean SL of 2.9 (Figure 4), which is significantly lower than the mean SL of the 3.8 high nitrogen group in the aphid-free arugula plants.

Additionally, the infected high nitrogen group has a significantly higher mean SL of 3.8 than the infected control group's SL of 3.2. These results indicate that aphid herbivory increases once a high nitrogen level in arugula is reached.

Fertilizer and Aphid Presence on Arugula Growth

WITHOUT APHIDS
The results, depicted in (Figure 5), indicated that in plants not infected with aphids, over time, mean mesophyll leaf count decreased across all groups. However, it was particularly significant that the high potassium treatment group experienced the largest decrease in average leaf count of 60% by the conclusion of the experiment. The recommended potassium group had a significant 260% increase in leaf count on day 6. These findings propose that a high potassium treatment is detrimental for arugula growth, but a recommended level of potassium promotes arugula growth. No other significant interaction was observed between the remaining groups (high nitrogen, recommended nitrogen and control).

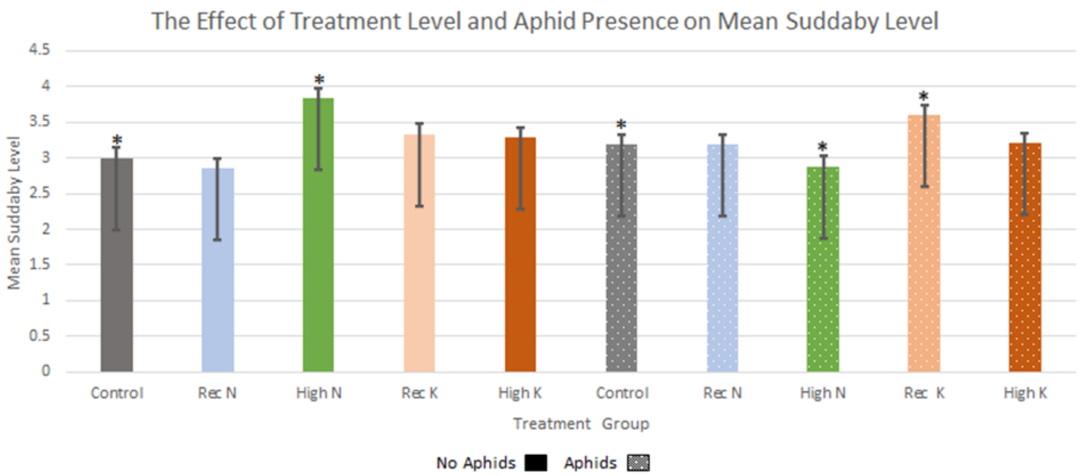

Figure 4: The average Sudabby Level for five treatments (control, high or recommended nitrogen, and high or recommended potassium) with no aphids over time(± 0.142SE). The absence of aphids is indicated by solid colours) and aphid presence (dotted bars). To create this plot, a two-way ANOVA examining the effects of treatment and aphid presence was completed ($F_{4,350} = 7.346$, $P = 1.086e\text{-}05$). An Estimated Marginal Means test was then performed to determine the following significant effects: between the high nitrogen group with no aphids and recommended nitrogen group, the high nitrogen group (no aphids) and control plants (no aphids), the recommended potassium (with aphids) than the high nitrogen (with aphids), and the high nitrogen group (with aphids) and high nitrogen group (no aphids).

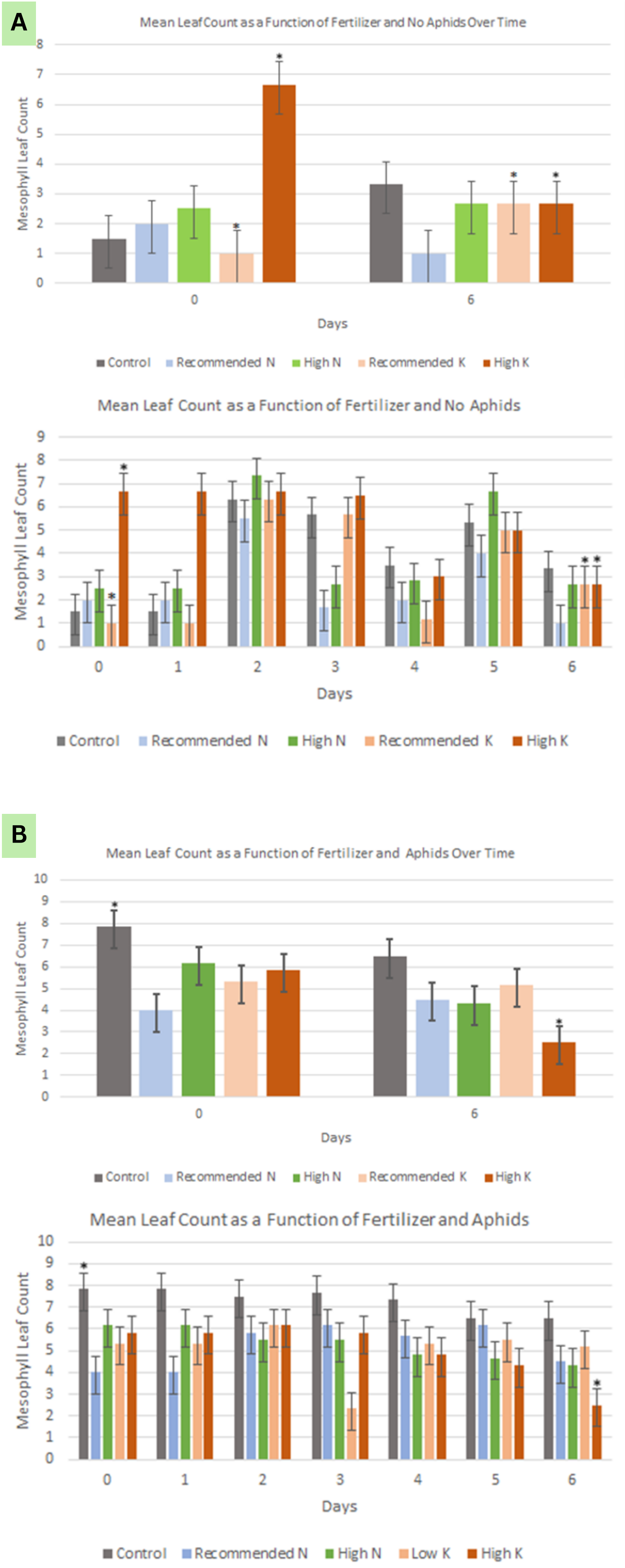

Figure 5: The mean mesophyll leaf count for all treatment groups over time (± 0.753SE), with aphids (a) and no aphids (b). To create this plot, a threeway-way ANOVA examining the effects of treatment and time and aphid presence was completed ($F_{24,350}$ = 7.346, P =7.604e-05). An Estimated Marginal Means test was then performed to determine the following significant effects in this study: (a) the potassium treatment group on days 0 and 6, the recommended potassium treatment on days 0 and 6, (b) the control group on day 0 and 6). Below Figure 5a and 5b are the effects of the treatments and aphid presence on mesophyll leaf count on each day.

WITH APHIDS

Within the plants infected with aphids, there was no significant change in the mesophyll leaf count of the high potassium, recommended potassium, high nitrogen, recommended nitrogen and control group count over time. Additionally, on day 6, the control group had a statistically insignificant mean leaf count 60% larger than the high potassium group (Figure 5). These findings suggest that aphid herbivory has no statistically significant effect on the four arugula treatment groups and control groups. Moreover, it also indicates that aphid herbivory is higher in plants with a high level of potassium with respect to the control.

DISCUSSION

Effect of Potassium and Nitrogen-Based Fertilizer on Aphid Population Growth

In summary, it was discovered that the performance of green peach aphids is impacted by the alteration of nitrogen and potassium concentration in soil. There were no significant differences between the use of potassium or nitrogen-based fertilizers and the control treatment. However, a significant difference was observed in green peach aphid abundance between the use of the recommended dose of nitrogen fertilizer compared to both the recommended and higher doses of potassium fertilizer (Figure 2). In general, nitrogen fertilizer was found to facilitate greater population abundance of green peach aphids, while potassium fertilizer was found to impede green peach aphid population growth.

The increase in green peach aphid population growth due to nitrogen fertilizer use can be attributed to the growth of the arugula plant (Rousselin et al., 2016). Green peach aphid abundance has been strongly linked to plant growth, and nitrogen fertilizer is an integral component of arugula growth and development (Rousselin et al., 2016). As a result of the addition of nitrogen-based fertilizer, above-ground vegetative growth is promoted, including the flush of new leaves, providing a preferred habitat for green peach aphids (Sauge, Grechi, and Poessel, 2010). These results support the plant vigour hypothesis, which contends that herbivorous insects perform better on vigorously growing plants (Sauge, Grechi, and Poessel, 2010). However, it was also observed that overuse of nitrogen fertilizer produces a smaller mean aphid count compared to recommended doses of nitrogen, suggesting excessive nitrogen levels can limit green peach aphid population growth rates (Zehnder and Hunter, 2009). Green peach aphids possess an elemental threshold ratio, that once passed, may lead to a decrease in overall population growth (Zehnder and Hunter, 2009). The higher-than-recommended nitrogen doses may have caused this event to occur.

Contrarily, the two potassium-based treatments produce the smallest mean aphid counts of the study (Figure 2). This result corroborates that of Sarwar, Ahmad and Tofique (2011), a study exploring the effect of potassium on aphid populations. A potential reason for the lower aphid populations in these treatments is that the higher potassium nutrition for the arugula diminished the concentration of nitrogen available through the phloem to aphids, which as a result, decreased their abundance (Sarwar, Ahmad, and Tofique, 2011). These results validate the theory that crop fertilizers can be used to control pest populations through the modification of the insect's food (Sarwar, Ahmad, and Tofique, 2011). Due to the use of potassium-based fertilizers, green peach aphids become nitrogen-limited, causing aphid populations to reduce through a bottom-up effect (Walter and Difonzo, 2007). Taken together, these findings demonstrate that through the manipulation of fertilizer content, farmers can control pest density and potentially improve crop productivity (Sarwar, Ahmad, and Tofique, 2011).

Leaf Count and Plant Condition on Aphid Count

Leaf count was observed to have a significant relationship with the aphid count of a plant (Table 1). As a general trend, as the leaf count of a plant increases, so does the mean aphid count.

Further, the mean aphid count of plants with eight leaves was significantly higher than any other plant (Figure 1). This trend was expected to occur and agrees with the results of Kumar (2019) as well as Morais et al. (2020). This is because an increased leaf count and leaf area provides more space for the aphids to live, meaning each plant can support a greater number of aphids. Additionally, aphids also specialize in ways that allow them to exploit periods of rapid plant growth. Aphids exhibit parthenogenetic viviparity, meaning that they can reproduce asexually and produce a live clone (Kumar, 2019). This allows them to reproduce rapidly and sustain high numbers on plants experiencing growth.
However, it was predicted that SL would have an influence on aphid count and yet this did not occur. It was anticipated that aphids would gravitate towards plants with a low SL and avoid those with a high SL (Marantos, 2020). This is because a high SL value implies that a plant has received sufficient resources, such as water, sunlight, and nutrients, and is therefore capable of persisting (Marantos, 2020). On the other hand, weaker plants (with a lower SL value) are themselves inept at sustaining the functions necessary for growth, leaving them vulnerable to infestations (Marantos, 2020).

One possible cause of this discrepancy is that the localized damage caused by aphid feeding is insufficient to cause a significant effect on the SL after just 13 days. The primary visual symptom of aphid infection is localized chlorosis and tissue damage at and around the feeding site, due to the toxic effect of aphid saliva on host plants (Kumar, 2019). Aphids are also capable of indirectly causing a significant amount of damage to host plants through the transmission of phytopathogenic viruses, although this is not expected to have occurred in this experiment (Kumar, 2019). It is likely that if the experiment had continued for longer, the damage caused by the aphids may have reached a significantly detectable level.

Unexpected damage to the plants during data collection may also account for the lack of an expected significant effect of SL on aphid population growth. While observational data on the plants was being collected, it was noted that multiple leaves appeared to have snapped stems. It was determined that this was caused by leaves becoming trapped under the lid of the tray due to the lid being frequently replaced during data collection. This damage was relatively consistent across groups. At this stage, it cannot be ascertained which of these confounding factors resulted in the absence of a trend, and thus further experimentation is required.

Fertilizer Presence on Arugula Condition

WITHOUT APHIDS

In the plants without aphids, all treatments other than the recommended level of nitrogen produced a higher mean SL than the control (Figure 4). Of these, the high nitrogen group was noted to be significantly different from the control (Table 1). It is well-established within the existing literature that nitrogen is highly absorbed by arugula and that it is an essential macronutrient to the plant (Morais et al., 2020). Nitrogen availability is frequently attributed as a limiting factor, impacting arugula growth and condition more than any other nutrient. Not only is nitrogen a key component of many proteins in arugula, but it is also a major component of the chlorophyll molecule (Morais et al., 2020). Arugula has also been shown to have a significantly higher nitrate content than other leafy vegetables, at concentrations of 4354.9mg/kg (Brkić et al., 2017). Potassium is also a key nutrient to the condition of arugula, as it plays a role in enzyme activation, photosynthesis and protein synthesis (Morais et al., 2020). When either nitrogen or potassium are at insufficient levels, these fundamental processes are interrupted causing a decline in plant condition. This study did not find a significant difference between the mean SL for each fertilizer treatment and only high nitrogen showed a significant difference from the control.

One treatment that was noted to have a lower mean SL than the control was the recommended nitrogen level. It should be noted that this treatment did not demonstrate a significant difference from the control. Though exact values vary, most sources suggest that concentrations of 100 kg/hectare to 140 kg/hectare are ideal for arugula growth (Oregon State University, 2010) and in this study, the recommended dose of nitrogen was approximately equal to 95 kg/hectare. There are several potential explanations for this result, nonetheless. For instance, fertilizer doses are given in kilograms of fertilizer per hectare of farmland, which is not directly comparable to a single plant in a very small pot. It is possible that because this conversion prevents total accuracy, the amount of fertilizer dispersed into the soil and available for uptake by the plant does not match the recommended concentration.

WITH APHIDS

The various treatment groups showed different mean SL of plants with aphids when compared to plants without aphids. Mean SL increased in the presence of aphids in the control, recommended nitrogen and recommended potassium groups from their respective groups without aphids. Furthermore, both the control and high nitrogen groups with aphids had a significantly lower mean SL than the high nitrogen group without aphids. Within all the treatment groups that contained aphids, the recommended potassium groups had a significantly higher mean SL than the high nitrogen group. This indicates that aphid herbivory increases when plants reach a high level of nitrogen. As explained previously, nitrogen fertilizer facilitates greater population abundance. Although this study was ultimately inconclusive on the relationship between plant condition and aphid population, it is reasonable to say that a large increase in aphid population, as occurred in the high nitrogen group, would result in increased damage to arugula and thus a decrease in overall plant condition (Capinera 2001).

Effect of Potassium on Arugula Growth

Arugula, like many plants, requires potassium as an essential nutrient to promote protein synthesis, enzyme activation, and metabolism, all of which are vital to plant growth, reproduction and defence (Rani et al., 2021). However, in this study, the application of recommended potassium (K2O) treatment, as recommended by (Oregon State University, 2010), resulted in a 260% increase in leaf count over the duration of the study. The rising increase in leaf count can be attributed to potassium's key role in modulating chlorophyll and carotenoids (Naciri et al. 2021). These compounds are vital as they function as photocatalysts to propagate the process of photosynthesis (Naciri et al., 2021). Additionally, potassium mitigates the effects of cadmium (a metal often found in plants), which reduces carbon fixation and nutrient uptake by promoting root growth to increase nutrient uptake (Naciri et al., 2021). The results of this study align with a study done by Rani et al. (2021), who concluded that adding a recommended potassium level, improves plant growth and foliage count.

Furthermore, in this study, it was demonstrated that without herbivore pressure (Figure 5a), the high potassium treatment has a significantly (Table 1) detrimental effect on arugula growth by the conclusion of experiment on day 6 (Figure 5a). Initially, the high potassium plants on day 0 had a mean mesophyll leaf count of 6.67 but by day 6 it had dwindled to 2.67, a 60% decrease in leaf count. Such a large drop in leaf count indicates that the high potassium level is inhibiting the biochemical and physiological processes necessary for plant growth. Xu et al. (2020) confirmed this in their study, finding that an excessive potassium level in plants inhibited carbon fixation in leaves and impeded products of photosynthesis from reaching plant roots.

Additionally, with herbivore pressure, on day 6, the high potassium group still had a significantly lower mesophyll leaf count than the unfertilized control group (Figure 5b). Initially, this may imply that the decrease in leaf count is solely based on the harmful effects of high potassium fertilizer on arugula. However, a study done by Wooldridge and Harrison (1968) discovered that a higher potassium concentration as fertilizer increased the aphid population growth rate. Thus, it can be concluded that the decrease in leaf count of high potassium arugula plants can be attributed to the detrimental effects of a high concentration potassium fertilizer on plant growth, in addition to aphid herbivory.

Effect of Potassium on Arugula Growth

Contrary to initial expectations, it was determined that both high nitrogen and recommended nitrogen treatments had no statistically significant effects (Table 1) on arugula growth between the first and last day (Figure 5b). Nitrogen is an essential macronutrient in plants and is responsible for regulating plant growth and the production of allelochemicals (Sun et al., 2019). Hence, nitrogen plays a key role in protein synthesis as well as chlorophyll and nucleic acid production. Arugula, in particular, is often very responsive to nitrogen nutrient supply, and a study done by Silva et al. (2021) indicates that increasing levels of nitrogen content in fertilizer increases leaf count and improves overall plant growth.

A potential explanation for why nitrogen did not promote significant arugula leaf growth over time can be explained by plant nutrient demands. Arugula has a particularly high nitrogen demand in its second or third week (rapid growth stage) (Yang et al., 2021). During this time approximately 50-90% of nitrogen moves from leaves to the stem to begin seed development. However, this experiment occurred after six weeks during the mature stage of a plant's lifecycle (Yang et al., 2021). At this stage, the potentially high residual nitrate levels in the soil, prior to the treatment may have been enough to sustain arugula and as a result did not increase leaf count significantly as indicated by Hall et al. (2012).

LIMITATIONS

Despite these intriguing findings, there are several confounding variables within the experiment that may have altered the data. Namely, the contents of the soil may act as a confounding variable. In this study, the soil selection was predetermined so the impacts of its components on arugula growth cannot be determined. Additionally, the tightly packed arrangement of the samples within tray one and tray two could have facilitated the migration of aphids from one arugula plant to another. Unless there was more space available to adequately separate each sample inoculated with aphids, it would not be possible to prevent this migration from occurring. Finally, not all arugula plants were in the same condition at the beginning of the experiment. This may serve as a confounding variable because initial state consistency in studies such as these is highly important. If this investigation were to be reproduced, arugula plants that score a four on the SL should be selectively chosen.

In conclusion, this study demonstrates that the manipulation of potassium and nitrogen-based fertilizers can be used to regulate pest density and improve crop productivity. It was found that a recommended dose of nitrogen fertilizer is linked to an increase in aphid population and little improvement in arugula growth and condition. It was also found that a high dose of nitrogen fertilizer is linked to decreases in aphid population, compared to the recommended nitrogen level, and supports the improvement of arugula condition. This indicates that a higher application of nitrogen fertilizer will prevent aphid herbivory on arugula and that an insufficiency of nitrogen fertilizer will only increase plant herbivory and reduce crop yield. Furthermore, a recommended dose of potassium is linked to a decrease in aphid population but promotes arugula growth. Conversely, a high potassium treatment reduces aphid population and is detrimental for arugula growth. Such an effect suggests that potassium application in arugula should be maintained at a recommended dose and that overapplication will have no significant effect on aphid population, and instead reduce arugula crop yield. These results are indeed significant and can be used as future guidance on nitrogen and potassium fertilizer application on arugula.

REFERENCES

Brkić, D., Bošnir, J., Bevardi, M., Bošković, A.G., Miloš, S., Lasić, D., Krivohlavek, A., Racz, A., Ćuić, A.M. and Trstenjak, N.U., 2017. Nitrate in Leafy Green Vegetables and Estimated Intake. *African Journal of Traditional, Complementary, and Alternative Medicines*, 14(3), pp.31–41. https://doi.org/10.21010/ajtcam.v14i3.4.

Capinera, J.L., 2001. *Green peach aphid.* [online] University of Florida. Available at: <https://entnemdept.ufl.edu/creatures/veg/aphid/green_peach_aphid.htm> [Accessed 24 Oct. 2021].

Cleland, E.E., 2011. Biodiversity and Ecosystem Stability. *Nature Education Knowledge*, 3(10), p.14.

Cooil, B.J., 1948. Potassium Deficiency and Excess in Guayule L. Growth Responses and Mineral Content. *Plant Physiology,* 23(3), pp.286–308. https://doi.org/10.1104/pp.23.3.286.

Elferink, M. and Schierhorn, F., 2016. Global Demand for Food Is Rising. Can We Meet It? *Harvard Business Review*. [online] 7 Apr. Available at: <https://hbr.org/2016/04/global-demand-for-food-is-rising-can-we-meet-it> [Accessed 24 Oct. 2021].

Grangeiro, L., Oliveira, F.C.L., Negreiros, M.Z., Marrocos, S.T.P., Macedo de Lucena, R. and Oliveira, R.A., 2011. Growth and accumulation of nutrients in coriander and arugula. *Revista Brasileirade Ciencias Agrarias*, 6(1), pp.11–16. https://doi.org/10.5039/agraria.v6i1a634.

Grechi, I., Hilgert, N., Sauphanor, B., Senoussi, R. and Lescourret, F., 2010. Modelling coupled peach tree–aphid population dynamics and their control by winter pruning and nitrogen fertilization. *Ecological Modelling*, 221(19), pp.2363–2373. https://doi.org/10.1016/j.ecolmodel.2010.06.015.

Hall, M.K.D., Jobling, J.J. and Rogers, G.S., 2012. Factors Affecting Growth of Perennial Wall Rocket and Annual Garden Rocket. *International Journal of Vegetable Science*, 18(4), pp.393–411. https://doi.org/10.1080/19315260.2012.660565.

Kissel, David.E. and Harris, G., n.d. *Field Crops.* [online] University Of Georgia Extension. Available at: <http://aesl.ces.uga.edu/publications/soil/cropsheets.pdf> [Accessed 18 Sep. 2021].

Kumar, S., 2019. Aphid-Plant Interactions: Implications for Pest Management. In: M. Oliveria, ed. 2020. Plant Communities and Their Environment. IntechOpen. Ch.7.

Little, A.G., Arellano, C., Kennedy, G.G. and Cardoza, Y.J., 2011. Bottom-up effects mediated by an organic soil amendment on the cabbage aphid pests Myzus persicae and Brevicoryne brassicae. *Entomologia Experimentalis et Applicata,* 139(2), pp.111–119. https://doi.org/10.1111/j.1570-7458.2011.01112.x.

Liu, C.-W., Sung, Y., Chen, B.-C. and Lai, H.-Y., 2014. Effects of Nitrogen Fertilizers on the Growth and Nitrate Content of Lettuce (Lactuca sativa L.). *Int. J. Environ. Res. Public Health,* 11(4), pp.4427–4440. https://doi.org/10.3390/ijerph110404427.

Long, L. and Riedl, H., 1993. *Green Peach Aphid* | WSU Tree Fruit | Washington State University. [online] Available at: <http://treefruit.wsu.edu/crop-protection/opm/green-peach-aphid/> [Accessed 22 Oct. 2021].

Macdonald, M., 2017. *About Arugula.* [online] West Coast Seeds. Available at: <https://www.westcoastseeds.com/blogs/garden-wisdom/about-arugula> [Accessed 24 Oct. 2021].

Marantos, J., 2020. *Certain plants are susceptible to pests.* How to ID and end an infestation. [online] Los Angeles Times. Available at: <https://www.latimes.com/lifestyle/story/2020-02-28/plants-attract-pests-stressed-unloved> [Accessed 24 Oct. 2021].

Morais, E.G. de, Freire, M.M., Santos, A.Y. de O., Silva, G.G.C. da, Oliveira, E.M.M., Franco, L.B. and Souza, G.L.F. de, 2020. Growth and accumulation of macronutrients in arugula. *International Journal of Advanced Engineering Research and Science*, 7(9), pp.176–183. https://doi.org/10.22161/ijaers.79.20.

Naciri, R., Lahrir, M., Benadis, C., Chtouki, M. and Oukarroum, A., 2021. Interactive effect of potassium and cadmium on growth, root morphology and chlorophyll a fluorescence in tomato plant. *Scientific Reports*, 11(1), p.5384. https://doi.org/10.1038/s41598-021-84990-4.

Ogran, A., Conner, J., Agrawal, A.A. and Barazani, O., 2020. Evolution of phenotypic plasticity: Genetic differentiation and additive genetic variation for induced plant defence in wild arugula Eruca sativa. *Journal of Evolutionary Biology*, 33(2), pp.237–246. https://doi.org/10.1111/jeb.13558.

Ondrasek, G., Bakić Begić, H., Zovko, M., Filipović, L., Meriño-Gergichevich, C., Savić, R. and Rengel, Z., 2019. Biogeochemistry of soil organic matter in agroecosystems & environmental implications. *Science of The Total Environment,* 658, pp.1559–1573. https://doi.org/10.1016/j.scitotenv.2018.12.243.

Oregon State University, 2010. *Eruca vesicaria ssp. sativa.* [online] College of Agricultural Sciences. Available at: <https://horticulture.oregonstate.edu/oregon-vegetables/arugula-0> [Accessed 23 Sep. 2021].

R Core Team, 2021. R: *A Language and Environment for Statistical computing*. Available at: <https://www.R-project.org/.>.

Rani, P., Saini, I., Singh, N., Kaushik, P., Wijaya, L., Al-Barty, A., Darwish, H. and Noureldeen, A., 2021. Effect of potassium fertilizer on the growth, physiological parameters, and water status of Brassica juncea cultivars under different irrigation regimes. *PLoS ONE*, 16(9), pp.e0257023–e0257023. https://doi.org/10.1371/journal.pone.0257023.

Rousselin, A., Sauge, M.-H., Jordan, M.-O., Vercambre, G., Lescourret, F. and Bevacqua, D., 2016. Nitrogen and water supplies affect peach tree - green peach aphid interactions: the key role played by vegetative growth. *Agricultural and Forest Entomology,* 18(4), pp.1–8. https://doi.org/10.1111/afe.12168.

Santos, R.C. dos, Fokar, M., Romagnoli, E.M., Aziz, M., Bento, J.M.S. and Paré, P.W., 2021. Monitoring a beneficial bacterium (Bacillus amyloliquefaciens) in the rhizosphere with arugula herbivory. *Rhizosphere*, 18, p.100347. https://doi.org/10.1016/j.rhisph.2021.100347.

Sarwar, M., Ahmad, N. and Tofique, M., 2011. Impact of Soil Potassium on Population Buildup of Aphid (Homoptera: Aphididae) and Crop Yield in Canola (Brassica napus L.) Field. *Pakistan J. Zool*, 43(1), pp.15–19.

Sauge, M.-H., Grechi, I. and Poessel, J.-L., 2010. Nitrogen fertilization effects on Myzus persicae aphid dynamics on peach: vegetative growth allocation or chemical defence? *Entomologia Experimentalis et Applicata*, 136(2), pp.123–133. https://doi.org/10.1111/j.1570-7458.2010.01008.x.

Silva, P.A. da, Kinjo, S., Melo, M.P.B.X. de and Sala, F.C., 2019. Evaluation of arugula cultivars and seed production in the organic system. J*ournal of Seed Science,* 41, pp.423–430. https://doi.org/10.1590/2317-1545v41n4218457.

Silva, P.H.S., Cecílio Filho, A.B., Reis, I.D.S., Reyes, S.M.R. and Cruz, M.C.P.D., 2021. Nitrogen Rates on Growth, Yield and Nitrate Foliar Content of Arugula. *Revista Caatinga*, 34, pp.380–387. https://doi.org/10.5539/jas.v11n2p385.

Suddaby, T., Alhussaen, K., Daniel, R. and Guest, D., 2008. Phosphonate alters the defence responses of Lambertia species challenged by Phytophthora cinnamomi. *Australian Journal of Botany*, 56(6). https://doi.org/10.1071/BT07228.

Sudderth, E.A. and Sudderth, E.B., 2014. Quantifying aphid behavioral responses to environmental change. *Entomologia Experimentalis et Applicata*, 150(1), pp.7–18. https://doi.org/10.1111/eea.12139.

Tripodi, P., Francese, G. and Mennella, G., 2017. Rocket salad: crop description, bioactive compounds and breeding perspectives. *Advances in Horticultural Science,* 31(2), pp.107–114. https://doi.org/10.13128/ahs-21087.

Walter, A.J. and Difonzo, C.D., 2007. Soil Potassium Deficiency Affects Soybean Phloem Nitrogen and Soybean Aphid Populations. *Environmental Entomology*, 36(1), pp.26–33. https://doi.org/10.1603/0046-225X(2007)36[26:SPDASP]2.0.CO;2.

Wooldridge, A.W. and Harrison, F.P., 1968. Effects of Soil Fertility on Abundance of Green Peach Aphids on Maryland Tobacco. *Journal of Economic*

Entomology, 61(2), pp.387–387. https://doi.org/10.1093/jee/61.2.387.

Xu, X., Du, X., Wang, F., Sha, J., Chen, Q., Tian, G., Zhu, Z., Ge, S. and Jiang, Y., 2020. Effects of Potassium Levels on Plant Growth, Accumulation and Distribution of Carbon, and Nitrate Metabolism in Apple Dwarf Rootstock Seedlings. *Frontiers in Plant Science*, 11, p.904. https://doi.org/10.3389/fpls.2020.00904.

Yang, T., Samarakoon, U., Altland, J. and Ling, P., 2021. Photosynthesis, Biomass Production, Nutritional Quality, and Flavor-Related Phytochemical Properties of Hydroponic-Grown Arugula (Eruca sativa Mill.) 'Standard' under Different Electrical Conductivities of Nutrient Solution. *Agronomy*, 11(7), p.1340 https://doi.org/10.3390/agronomy11071340

Zehdner, C.B. and Hunter, M.D., 2009. More is not necessarily better: the impact of limiting and excessive nutrients on herbivore population growth rates. *Ecological Entomology*, 34(4), pp.535–543. https://doi.org/10.1111/j.1365-2311.2009.01101.x.