

Effects of Soil Salinity on Green Peach Aphid and Arugula Interactions

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Abstract

Myzus persicae (green peach aphid) may become a serious threat to global agriculture as a result of climate change. Rising global temperatures are increasing soil salinity for all environments, hampering agricultural output and general plant life. This study investigated the effects of soil salinity by administering NaCl solutions of varying concentrations to *Eruca vesicaria* (arugula), comparing aphid colonies hosted by these groups. A relationship was demonstrated between the concentration of NaCl administered, aphid colonies and plant condition. This was further expanded to find a clear positive correlation of leaf count and proportional aphid population change, regardless of time and treatment. This supports the claim that soil salinity and proportional aphid population change have an indirect negative, concentration-dependent relationship. Thus, the chief determinant of proportional population change is plant condition. This shows that concerns related to the plant stress hypothesis and increasing aphid damage on agriculture appear unfounded. In fact, this study supports the plant vigour hypothesis, suggesting green peach aphid is a species of flush feeder.

1 Introduction

The human population is growing rapidly, as is the demand for food (Shrivastava & Kumar 2015). Global agricultural output is predicted to need to increase by 25-70% by 2050 to meet these demands (Hunter *et al.* 2017). The Intergovernmental Panel on Climate Change has highlighted that changing temperatures and weather patterns are already hampering agricultural output (“Chapter 5: Food Security — Special Report on Climate Change and Land” 2024). One of the largest factors behind reduced output is increasing soil salinity. A factor which will continue to do so with increasing severity as climate change worsens (Mukhopadhyay *et al.* 2021; Qadir *et al.* 2010). This is the result of altered precipitation patterns due to climate change creating periods without sufficient rain to dissolve salts in the soil. In response to lowered precipitation, irrigation with saline water is being used; however, this is leading to the salinization of new, previously arable soil (Eswar *et al.* 2021; Shrivastava & Kumar 2015). Among salts accumulating in soil, NaCl is of note because both Na⁺ and Cl⁻ have been shown to be toxic to plants, as well as being one of the most abundant salts in soil (Alharbi *et al.* 2022; Flowers *et al.* 2015; Yan *et al.* 2015). When exposed to NaCl, absorption of water in the plant is weakened, leading to severe dehydration (Shrivastava & Kumar 2015). Na⁺ competitively inhibits the absorption of K⁺, which is a growth-limiting plant nutrient in many situations and a key component of commercial fertiliser (Wakeel 2013). This presents a barrier to other conventional methods of improving agricultural yield. Further, Na⁺ and Cl⁻ both interfere with physiological plant processes including photosynthesis and reproductive

development, which harm overall plant condition and agricultural yield (Hocking 1985). Thus, NaCl was chosen for this experiment due to its toxicity to plants and its abundance in most soil.

Another threat to agricultural yield that must be addressed is plant pests. Specifically, we will focus on *Myzus persicae* (green peach aphid), a model organism for understanding pest dynamics in agricultural ecosystems. Green peach aphid (GPA) is a notable pest for several crops, causing severe economic losses annually (Bos *et al.* 2010; Simon & Peccoud 2018). Green peach aphid is a parthenogenetic species of sap-sucking insect. Although this is detrimental to plant condition, the majority of the damage is the result of aphids being vectors for numerous viruses (Simon & Peccoud 2018). Some evidence has shown that soil salinity can potentially increase, or decrease aphid population growth, independently of the effects on plants (Eichele-Nelson *et al.* 2018; Ma *et al.* 2021). Our study aims to investigate how soil salinity-induced changes in soil chemistry may influence GPA populations and what this means for agricultural pest management in a changing climate. The research will examine how various levels of soil salinity impact aphid population growth rate and plant condition. In addition, we will investigate the interactions between aphid populations and host plants under saline conditions, using *Eruca vesicaria* (arugula) as a host plant. It stands to reason that as new land becomes salinized, it could increase the severity of GPA infestations. By examining these dynamics in a controlled laboratory setting, we aim to provide insights into how climate-driven salinization may influence pest outbreaks, crop vulnerability, and the overall conditions of agricultural ecosystems in the coming decades.

Our study seeks to answer (1) How soil salinity impacts GPA proportional population change (PPC) (2) If there is an effect, is it concentration-dependent (3) If there is an effect, is it direct (salt to aphid) or indirect (salt to plant to aphid).

We hypothesise that increasing soil salinity will increase the GPA population relative to available plant resources. We reviewed literature surrounding aphid species across two genera, where species-specific responses to soil salinity were observed and then compared to GPA for physiological and behavioural similarities. *Aphis gossypii* (cotton aphid) populations struggle in saline environments, likely due to their lower physiological tolerance for osmotic stress (Ma *et al.* 2021). Contrarily, *Aphis glycines* (soybean aphids) populations can thrive and grow in an environment even where plant condition suffers (Eichele-Nelson *et al.* 2018). Following the plant stress hypothesis, senescence feeders are predicted to benefit from feeding on plants undergoing additional stress as a result of their environment (White 2009). Green peach aphid has been shown to improve feeding under senescent conditions (Machado-Assefh *et al.* 2014). Accordingly, we predict that GPA will respond positively to increased soil salinity. Soil salinity stress in plants leads to an accumulation of osmolytes like soluble sugars and amino acids in the phloem sap, which phloem feeders such as GPA and soybean aphids can capitalise on to enhance their

reproductive rates (Wu *et al.* 2020). Studies suggest that this nutrient-rich sap provides a favourable environment for soybean aphids, leading to population surges in saline environments (Eichele-Nelson *et al.* 2018).

In contrast, cotton aphids demonstrate a lower tolerance for saline environments, likely due to differences in their osmotic regulation mechanisms and ecological adaptations. Cotton aphids may not be as well-equipped to cope with the physiological changes in host plants under salt stress, which could explain their population decline when exposed to higher soil salinity (Jiao *et al.* 2024). In fact, cotton aphids differ in that they perform worse in senescence conditions, behaving as flush feeders. Therefore, plants undergoing additional stress are inferior sources of food for them. This divergence in soil salinity tolerance within the *Aphis* genus underscores the importance of species-specific ecological interactions. Since *A. gossypi* and *A. glycines* are more genetically similar to each other than GPA, we rely on the plant stress hypothesis to predict that GPA, like soybean aphids, will display a robust capacity to adapt and even thrive in saline environments, relative to typical conditions.

2 Materials and Methods

2.1) Study system

Our experiment utilised 510 GPA, distributed evenly across 51 four-week-old arugula plants to investigate our research questions. The seeds for the arugula plants were obtained from William Dam Seeds and planted on August 20, 2024. Two seeds were placed in a pot with dimensions of 6.5cm x 6.5cm x 9cm, each filled with soil to a depth of 8 cm. The seeds were deposited approximately 1.5 cm deep into the soil. The soil was obtained from Holland Park Garden Gallery and no fertiliser was used. Each of the plants was grown under a light that had a 12-hour light cycle, and the plants were watered only when required. The GPA were obtained by Vineland Research & Innovation Centre and were housed on tobacco leaves before use. Our experiment was conducted over 11 days and took place in a controlled laboratory environment that maintained standard room temperature throughout the study. On day zero of the experiment, the arugula plants were partitioned into three groups of 17 and were placed in separate trays with closed lids. Tray lids had meshed openings to maintain ventilation while containing aphids within. 10 aphids were placed on each arugula plant, selected arbitrarily while excluding husks (entirely white, shrivelled aphid corpses). They were transferred using either forceps or a dissection probe.

2.2) Treatment methods

Aqueous NaCl treatments were administered to the two designated groups, in the respective high (2mL, 3.4M) and low doses (2mL, 1.7M), while the control group was left untouched. Chemical grade NaCl was

used as opposed to table salt for its lack of iodide content. If a commercial salt was used in the experiment, there would have been uncertainty in whether the NaCl or the iodide was the variable responsible for the change in aphid and leaf growth. Thus, iodide was identified as a potentially confounding variable and pure NaCl was used. Additionally, studies such as Kiferle *et al.*, have deduced that the administration of low concentrations of iodide to a plant can result in an increase in the growth and production of a plant, which would further confound our results (Kiferle *et al.* 2021). Low and high-concentration solutions of NaCl were prepared on day one and stored in a bottle to minimise evaporation over the course of the experiment. The solution was swirled to dissolve precipitates prior to administration.

The solution was injected into soil via a pipette with a pi-pump, in direct proximity to the roots. Dosing occurred on days one, four, and seven, with the same researcher administering the doses throughout the study for consistency. The plants were not dosed on day zero to ensure even spacing of doses across the experiment. The number of aphids appearing on each plant was counted on days one, four, seven, and 11. The number of leaves on each plant was also counted on these days as a quantitative metric of plant condition. Green peach aphid was counted systematically using a dissection probe to overturn leaves, starting with the top leaf and moving down the plant, counting the stem last, and using a mechanical counting device to keep track. Population proportional change was determined by dividing the aphid population on the current treatment day by the aphid population on the previous day.

2.3) Statistical Methods

By the Shapiro-Wilk test, all data groups that measured PPC and leaf count were not normally distributed. Therefore, the Kruskal-Wallis test, a non-parametric alternative to ANOVA was selected for analysis. *Post hoc* analysis of these groups was conducted using Dunn's test with Benjamini-Hochberg adjusted P-values. All statistical tests were conducted in R Statistical Software (v4.3.3; R Core Team 2024), with Dunn's test function sourced from the Simple Fisheries Stock Assessment Methods package (v0.9.5; Ogle *et al.* 2023).

2.3.1) Leaf count

Analyses of the impact of soil salinity on arugula leaf count were conducted. In the first analysis, leaf counts were grouped exclusively by treatment levels, with data sourced from the duration of the experiment. Four additional analyses were conducted, corresponding to each data collection day, with data grouped by treatment level in each day.

2.3.2) Population Proportional Change

This measurement was selected to measure growth relative to previous population, minimizing inherent bias associated with differing initial conditions. Population proportional change was analysed in a similar fashion to the leaf count data. In the first analysis, the population data was grouped exclusively by their treatment across the experiment. The subsequent analyses looked at the data corresponding to each interval (day one to day four, day four to seven, day seven to 11) comparing each treatment level on these intervals. Population proportional change was also compared within treatments, with respect to time.

2.3.3) Continuous analysis

The overall relationship between PPC and leaf count data was analysed using linear regression. Total population data was $\log(n+1)$ transformed.

3 Results

3.1) Population Proportional Change

Overall PPC were shown significantly lower with increased soil salinity (chi-square = 10.359, d.f = 2, $P < 0.01$). From day one to day four, there was no significant difference between the PPC for any of the treatment levels (chi-square = 3.750, d.f = 2, $P > 0.05$). Population proportional change from day four to day seven for weak and strong treatment groups were significantly lower than the control group ($Z = 3.721$, adj. $P < 0.001$), ($Z = 2.700$, adj. $P < 0.05$) respectively. Population proportional change on this interval was not significantly different between the weak and strong treatment groups ($Z = -1.021$, adj. $P > 0.05$). Population proportional change from day seven to day 11 of the strong treatment group was significantly lower than control, and weak treatment groups ($Z = 4.000$, adj. $P < 0.001$), ($Z = -3.991$, adj. $P < 0.0001$). However, the control group and the weak treatment group were not significantly different in this interval ($Z = 0.006$, adj. $P > 0.05$) (Figure 1, Figure 2). Under strong treatment, PPC significantly decreased over time (chi-squared = 17.720, d.f = 2, $P < 0.001$). Under weak treatment and control, PPC did not significantly change over time (chi-square = 4.705, d.f = 2, $P > 0.05$), (chi-square = 5.982, d.f = 2, $P > 0.05$). Total population changes are shown in Figure 3.

3.2) Leaf count

Arugula leaf count significantly decreased overall with increased soil salinity (chi-square = 14.154, d.f = 2, $P < 0.001$). Prior to salt exposure, arugula within the strong, weak, and control treatment group, did not have significantly different leaf counts (chi-square = 4.999, d.f = 2, $P > 0.05$). After 10 days of salt

exposure, arugula within the strong treatment group had significantly fewer leaves than control arugula ($Z = 4.533$, $\text{adj.P} > 0.0001$), and weak treatment group arugula ($Z = -3.180$, $\text{adj.P} < 0.005$). Arugula in the weak treatment group did not have significantly fewer leaves than control arugula ($Z = 1.353$, $\text{adj.P} < 0.05$).

3.3) Comparison of Leaf Count to Population Growth

Linear regression analysis of aphid population ($\log(n+1)$ transformed) to leaf count, and PPC to leaf count both yielded significant correlations ($\text{adj.R Squared}=0.305$, $P<0.0001$) and ($\text{adj.R Squared}=0.095$, $P<0.0001$) respectively (Figure 4).

3.4) Qualitative assessment

The qualitative assessment showed that aphids tend to accumulate on the ventral surface of large leaves or clustered at new growth. Aphids appeared to occupy healthier, more viable plants, avoiding decaying plant tissue. Healthier plants were qualified as plants with relatively more leaves, and little to no spotting or shrivelling (Figure 5). On day one, we also observed that aphids tended to cluster around growing leaves, with the large populations accumulating on the underside of the largest leaves. These spatial patterns exhibited by GPA were consistent between treatments and did not differ over time.

4 Discussion

In this study, we found evidence that GPA is impacted by soil salinity in a concentration-dependent manner (Figure 3). Population proportional change differed between treatments, particularly towards the end of the study after extended soil salinity exposure, where the control and weak groups saw 35% less growth. (Figure 1). This evidence suggests that as soil salinity increases, GPA PPC will decrease. Soil salinity likely decreases overall PPC through an unknown mechanism, in addition to limiting the population that can be supported by each plant. This interval-based analysis helped to investigate population dynamics over time. Evidence also suggests that PPC and total GPA population are significantly correlated with leaf count, which can be used as a metric of plant condition (Figure four). This points towards the GPA population being limited by plant condition in this situation as opposed to other known limiting factors, expressly soil salinity. Additionally, the differences between treatment groups throughout the study were similar for both leaf count and aphid count, particularly on day 11 (Figure 1). This indirect relationship between soil salinity on GPA dynamics is further supported by qualitative assessment. Within the strong and weak treatment groups, plants that appeared to have the best condition were capable of holding notable aphid populations (Figure 5). Although the largest populations

found on control plants far exceeded the largest on treated plants, these notable populations on treated plants indicate that plants of good condition rooted in saline soil could support notable GPA populations. Although evidence supports the claim that the effect of soil salinity on GPA is indirect, no explicit mechanism can be determined from these results, besides the previously established toxicity of Na⁺ and Cl⁻ to plants. NaCl toxicity to GPA in the same magnitude as NaCl toxicity to plants remains a possible, though improbable, alternative explanation for the observed results. Further studies must be conducted to determine the precise interaction of soil salts with GPA.

Due to the constraints of this study, NaCl was the only salt used. However, NaCl is not the only salt contributing to increased soil salinity, and this study could be replicated using different salts to further improve understanding of the agricultural implications of increasing soil salinity.

The proposed indirect relationship is contrary to previous research on different aphid species where a direct positive or negative relationship of soil salinity on aphids was demonstrated. Soybean aphids and cotton aphids both demonstrated clear relationships to the soil salinity of the soil in which their respective plants were grown, prompting the conclusion that GPA would as well (Eichele-Nelson *et al.* 2018; Ma *et al.* 2021).

From our study, concerns surrounding climate change worsening the agricultural damage accomplished by GPA appear to be unfounded. That is because as crop condition declines due to increasing evapotranspiration, altered precipitation patterns and increasing soil salinity, GPA populations will decline proportionally and their effect will remain constant relative to plant biomass. That said, a proportional decrease can still impose a large burden upon the agricultural sector and larger ecosystems when a resource is scarce. This calls for more research into reducing GPA population sizes and limiting mobility, to protect increasingly limited agricultural resources.

An important thing to consider is that any decrease in GPA populations does not imply a reduction in the overall threat they pose to crop health. Given that crops are already under stress from soil salinity and other climate-driven factors, a lower GPA population may still significantly impact weakened crops. Therefore, while GPA numbers may diminish, their potential to exacerbate the negative effects of other stressors remains. Additionally, our study focused on the interaction between GPA populations and soil salinity under controlled conditions that excluded predators and other biotic factors. In natural agricultural ecosystems, predator-prey dynamics may further influence GPA populations, potentially leading to more complex outcomes. Future research should incorporate these ecological interactions to better predict real-world outcomes in diverse environments. One limitation of our study was the inability to measure NaCl toxicity on GPA directly. While arugula plant conditions metrics such as leaf count provided evidence of

the indirect effects of soil salinity on aphid populations, future research should aim to isolate and quantify the direct physiological impacts, or lack thereof. This could involve separate assays where GPA are exposed to NaCl without arugula plant mediation, allowing for a more definitive conclusion on whether the observed population declines are due to direct toxicity or indirect effects mediated through arugula plant condition.

Although soil salinity initially stunted GPA population growth, in the last segment of the study the PPC and leaf count were not significantly different between the weak and control treatments. This evidence suggests that over time, arugula and/or GPA can adapt to an environment of somewhat augmented soil salinity. This raises questions concerning agriculture in an increasingly saline environment. If arugula plants are allowed to grow in slightly saline soil, it could lead to comparable GPA health which was shown to be correlated with arugula health, suggesting a greater resiliency of arugula to saline soil.

There was no evidence that soil salinity would increase the proportional agricultural losses to GPA as soil salinity was shown to curb population growth. The spread of GPA populations is largely due to the select adults of alate morphology that spread short distances to new plants (Taylor 1977). Certain spatial patterns of soil salinization could stand to stop aphids from reproducing in a particular region. Since GPA can only move short distances each generation, portions of highly saline soil and thus decaying plant health could stand as a barrier to virus transmission between different geographical regions.

Although GPA are traditionally regarded as agricultural pests, they can serve as analogues for insects and other organisms that depend on arugula and other crop plants, both for habitation or sustenance. The significant effect of soil salinity on arugula condition, and the significant effect of arugula condition on GPA, can be extended to other systems. Decreased plant condition as a result of soil salinity is not only consequential to anthropogenic food demands but also threatens the species that rely on them. Increasing soil salinity can be expected to damage the biodiversity of many ecosystems.

This study provides significant evidence that soil salinity affects both the GPA populations and arugula plant condition. Throughout the experiment, GPA populations were observed to decrease substantially under greater salt concentrations, which is supported by the experimental data of reduced leaf count in the low and high-dosage group. As arugula plant condition declined under greater soil salinity, they showed lowered ability to host aphid populations, which ultimately led to a decrease in their PPC. However, the reduction in GPA numbers observed in this study should not be misinterpreted as a complete alleviation of

pest pressure in saline environments. These findings do suggest that the mechanisms affecting aphid populations are likely linked to the overall condition of the host arugula plant. This understanding is vital as it states there is likely an indirect relationship between soil salinity stress on arugula plants and its impact on GPA PPC.

Furthermore, the study opens the door for future research into the direct physiological effects of NaCl on GPA, which was not fully explored in this experiment. While the results indicate that arugula plant condition mediates the impact of soil salinity on GPA populations, it remains unclear whether NaCl itself has a direct toxic effect on the aphids. Future studies should consider isolating GPA from their host arugula plants and exposing them to NaCl or other soil present salts, to better understand the interactive mechanism of soil salinity and agricultural pests. Such investigations would help clarify whether the observed population declines are primarily due to arugula plant-mediated effects or direct toxicity to the aphids themselves.

Our results supported the plant vigour hypothesis, while previously established literature supports the plant stress hypothesis (Machado-Assefh *et al.* 2014). Both hypotheses can both hold true along a spectrum of flush and senescence-feeding herbivores (White 2009). Aphids have previously been demonstrated to benefit from feeding under senescent conditions, but this study showed they benefit from increased plant availability, rather than senescence. This supports the Whites statement that aphids may benefit from an increase in the quality of food, but meaningful population growth can only be explained by an increase in the quantity of food (plant growth) (Machado-Assefh *et al.* 2014).

In conclusion, our results showed that GPA growth rates are impacted by soil salinity.

Furthermore, soil salinity impacts on PPC increased over the duration of the study. The effect is shown to be dependent on the magnitude of soil salinity, and can effectively be explained by an indirect mechanism, in which soil salinity reduces plant condition, which in turn reduces aphid populations.

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Tables and Figures

Figure 1 Mean GPA population per plant in the control, low soil salinity, and high soil salinity treatments, compared across days one, four, seven and 11 of soil salinity exposure. Values are means \pm SE.

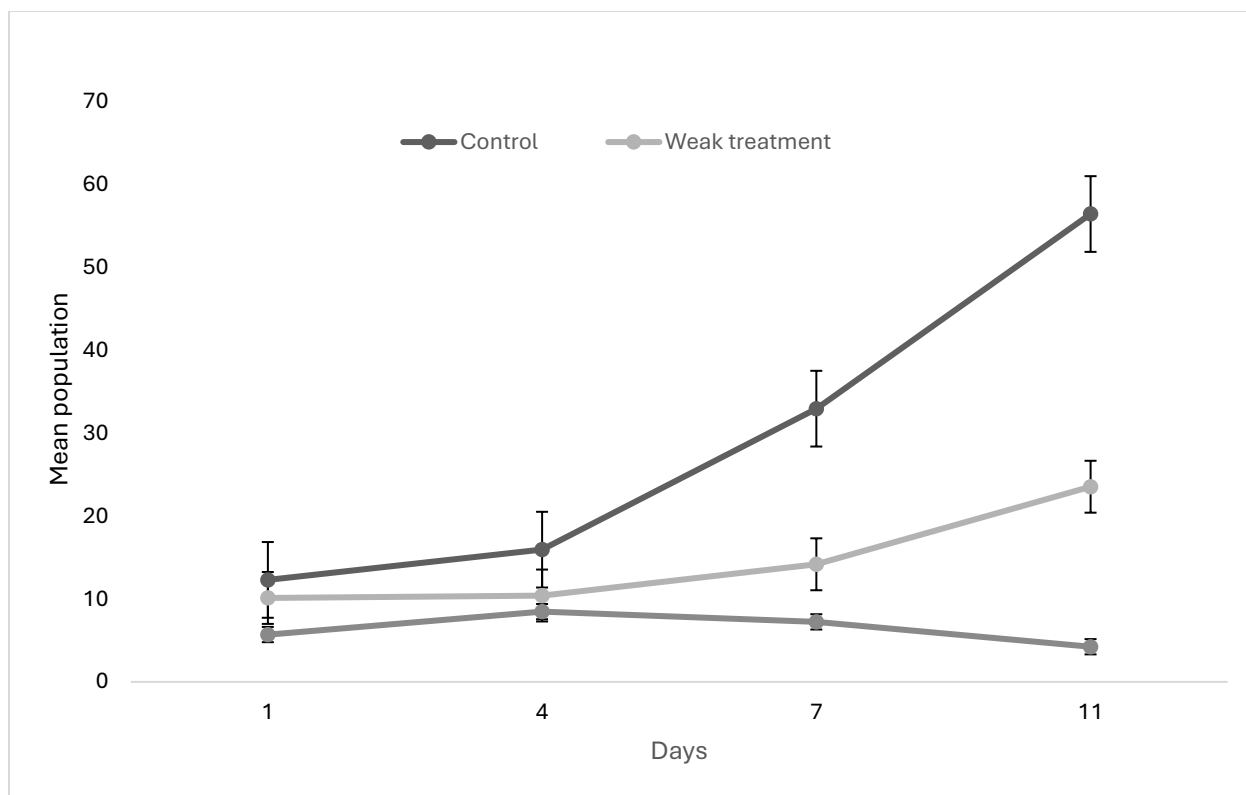
Figure 2 Statistical significance of various leaf count and PPC analyses between groups classified by soil salinity treatment level. Soil salinity showed an overall significant effect on both leaf count and PPC. Further, comparison between day 11 groups in salinity on both leaf count and PPC show similar patterns of difference.

Figure 3 Mean population proportional change (ie. population at day four \div population at day one) compared between control, low soil salinity and high soil salinity treatments, on the intervals of day one - day four, day four - day seven and day seven - day 11. Values are mean \pm SE. The difference between the control, low soil salinity and high soil salinity on each interval were compared using a Kruskal-Wallis

test, and the pairwise differences were compared using Dunn's *post hoc* test utilising the Benjamini-Hochberg P-value transform to determine significance. Letters denote significance only within the interval, and do not compare significance between intervals.

Figure 4 Relationship between the number of leaves and GPA count, $\log(n+1)$ transformed, including data from all treatment groups and the duration of the study. The trend line indicates a positive correlation between the variables.

Figure 5 Arugula plants in the strong soil salinity treatment group pictured on the final data collection day. Plants boxed in white possess more than two leaves and have been deemed in good condition by this metric. Those boxed in red possess two leaves or less, and have been deemed in poor condition. Aphid counts per plant have been overlaid. Certain plants maintain notable condition and aphid populations despite high soil salinity treatment.



Statistical Analysis of Salinity on Leaf Count and PPC			
Leaf Count		PPC	
Overall	Significant	Overall	Significant
Day 11			
Control - Strong	Significant	Control - Strong	Significant
Control - Weak	Insignificant	Control - Weak	Insignificant
Strong - Weak	Significant	Strong - Weak	Significant

