



Testing the effects of nitrogen on the interaction of *M. persicae* and *A. thaliana*

LIZ BALINT, LEENA ALSHENAIBER, NOEL KIM, ISOBEL SHARPE, MEGAN SWING

Integrated Science Program, Class of 2019, McMaster University

SUMMARY

Natural elements serve as the building blocks of ecosystems, and cycle through the biosphere. Nitrogen is one of the most important elements for plant growth. To further increase plant growth, nitrogen is artificially added to ecosystems as fertilizer, though it may put nearby organisms at risk. The impact of fertilizer runoff affects many environments and the organisms that inhabit them. For these reasons, it is important to understand the effects of increased amounts of nitrogen on plant-animal interactions. To do so, we studied the effect of varying ammonium nitrate (AN) concentrations, a compound commonly found in fertilizer, on the interaction between *Arabidopsis thaliana* and *Myzus persicae*. The control group *A. thaliana* plants were treated with water, while low and high dose groups were treated with varying concentrations of AN. We counted the number of *M. persicae* present on each *A. thaliana* plant throughout the study period. The low dose group begins to plateau after the sixth day, while the control and high dose groups grew. These results suggest that soil nitrogen content affects trophic interactions between plants and herbivores. The optimal treatment was a low dose of AN, as population growth of *M. persicae* plateaued, limiting herbivory and potentially benefiting *A. thaliana*.

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INTRODUCTION

NITROGEN AND PLANTS

Plants are the basis of ecosystems, and changes in plant growth have a substantial effect on said ecosystems (Maron and Crone, 2006). The rapidly increasing human population has led to an increased demand for plants used for food, oil, and energy (PRB, 2016). In order to fuel this demand, agriculturalists rely heavily on fertilizers such as inorganic nitrogen, or ammonium nitrate (AN). AN is readily absorbed by plants, as it has a positive charge that can be held by the abundant negatively charged ions in soil (Liu et al., 2014; UCAR, 2016). Nitrogen is an essential nutrient for plants due to its use in metabolic processes and plant structure. By extension, increased levels of nitrogen give plants

stronger defenses against herbivory (Sohlenkamp et al., 2002). Thus, it is commonly used in fertilizers. Despite its benefits, nitrogen fertilizer can also cause environmental damage through runoff from crops to surrounding biota (Liu et al., 2014). This affects plant growth, and can eventually alter plant-animal dynamics.

STUDY SYSTEM

Arabidopsis thaliana, known as thale cress, is a weed commonly used as a model organism due to its small genome and relatively short life span (Koornneef, Alonso-Blanco and Vreugdenhil, 2004). *Myzus persicae*, another model organism commonly known as the green peach aphid, is a generalist that feeds on the phloem of a wide variety of plants, including *A. thaliana* (Louis, Singh, and Shah, 2012). In general, plants employ a number of chemical and mechanical defenses to deter

this sort of herbivory. In our study system, *A. thaliana* has several defenses against the phloem-sucking herbivory of *M. persicae* (Kuśnierczyk et al., 2008).

PLANT DEFENSE

A. thaliana has a variety of characteristics used to defend itself against the herbivory of *M. persicae*. Defensive responses in the plant are elicited by the saliva secreted by *M. persicae* when sucking on the phloem of *A. thaliana*, as well as the penetration of the phloem cell wall itself (Miles, 1999; Kuśnierczyk et al., 2008). Components of *M. persicae* saliva lead to the accumulation of jasmonic acid in the plant, a compound known to transduce signals of attack throughout the plant (Kuśnierczyk et al., 2008). Salicylic acid signalling pathways are also induced during herbivory, although jasmonic acid pathways are more efficient. Due to antagonistic crosstalk between the two signalling pathways, *M. persicae* can induce salicylic acid signalling responses, thereby suppressing jasmonic acid signalling (Wees et al., 2000; De Vos, Kim and Jander, 2007). During herbivore feeding, Ca^{2+} membrane channels also open, allowing areas of the sieve element membrane that were broken by *M. persicae* to be plugged by proteins (Will and van Bell, 2006; Kuśnierczyk et al., 2008). *M. persicae* then attempt to prevent these defensive responses by injecting Ca^{2+} binding proteins into the sieve element membranes, which stops *A. thaliana*'s proteins from repairing membrane damage.

OUR STUDY

The interaction between nitrogen fertilizers and various plants has been studied, demonstrating that nitrogen addition to the soil affects some aspects of plant growth and development, but not others (Chechin and Fumis, 2004; Houle and Moore, 2008). Additionally, the interactions between *M. persicae* and *A. thaliana* have been studied extensively (De Vos and Jander, 2009; Kim and Jander, 2007). Beyond this, the impact of *M. persicae* on nitrogen metabolism in plants such as alfalfa has been explored by Girousse et al., 2005, who found that *M. persicae* infestation led to reduced nitrogen content and associated negative nitrogen deposition rates. Altered nitrogen availability influences the magnitude of response to herbivory by altering the levels of compounds such as jasmonic acid, which alert plants to biotic and abiotic stressors (Schmelz et al., 2003). However, the effects of nitrogen fertilizer on the interactions between *M. persicae* and *A. thaliana* have not been demonstrated. Subsequently, in this study we propose to investigate how varying amounts of nitrogenous compounds in the soil of *A. thaliana* affect the per capita growth rate of *M. persicae*. We used the pop-

ulation of *M. persicae* on *A. thaliana* to infer the potential effects of runoff water contaminated with nitrogen fertilizer on plant growth. We hypothesize that the *M. persicae* population will initially increase with higher concentrations of nitrogenous compounds available in the soil. Over time, we predict that *A. thaliana* will use the added nitrogen in the soil to strengthen its defenses against the increasing rates of herbivory.

MATERIALS AND METHODS

EXPERIMENTAL SETUP

This experiment was conducted over a 13-day period in a laboratory at McMaster University in Hamilton, Ontario during early autumn. 24 wild type (Col) *A. thaliana* plants were obtained in the rosette stage of growth. The plants received sunlight from a north-facing window and were kept at room temperature.

TREATMENT AND INOCULATION

The *A. thaliana* plants were randomly divided into three groups of eight: control, low dose, and high dose. Two concentrations of AN were created by dissolving solid crystal AN in water, with a concentration of 60 ppm for the low dose group and 300 ppm for the high dose group, which are realistic fertilizer runoff concentrations (Kim et al., 1986). Next, 12 mL of distilled water was added to the control plants, and 12 mL of the low and high dose AN solutions were added to the respective plants, using a pie pump. The AN was dissolved in water to allow for a faster uptake of nitrogen from soil to plant. The solution was added to the control group first, then to the low group, and lastly to the high group in order to avoid contamination of solutions by the pie pump. Using a teaser needle, each *A. thaliana* was inoculated with four *M. persicae*. This is a suitable number of *M. persicae*; even if one or two insects do not survive, there is still opportunity for population growth. The plants were labelled and placed into three large plastic trays which were covered with clear plastic lids to avoid external contamination and the transfer of *M. persicae*.

DATA COLLECTION

Data was collected on days 4, 5, 6, 7, 11, and 12 after inoculation. The number of *M. persicae* was counted for each plant by removing one pot at a time from the lidded containers to prevent any alates from escaping. To reduce human error, each plant was counted by two researchers, and the mean of the two counts was used for analysis. On each of these days, we also noted whether the plant was bolting and the number of leaves beginning to yellow per plant. Photographs of each of the

plants were also taken.

STATISTICAL ANALYSIS

All analyses were done using the statistical software, R (R, 2015). An analysis of covariance (ANCOVA) was performed on the mean *M. persicae* count, the treatment group (control, low dose, high dose), with a continuous predictor of time (measured in days). An interaction term between treatment and day on the mean *M. persicae* count was also analyzed in the ANCOVA. Plant H6 from day 12 was an outlier and removed from analysis.

Table 1: Summary of ANCOVA for the effect of treatment, day, and the interaction between treatment and day on mean *M. persicae* count over a 13-day period.

Mean <i>M. persicae</i> count				
	Df	Mean Sq	F value	Pr(>F)
Treatment	2	9.5	0.1160	0.89060
Day	1	6653.2	84.4438	1.454e-15
Treatment:Day	2	430.8	5.2731	0.006214
Residuals		11191.6	81.7	

RESULTS

The effect of time on mean *M. persicae* count was statistically significant ($p < 0.05$; Table 1). Furthermore, the interaction term between treatment and day was statistically significant (Table 1). The treatment as the sole factor did not have a statistically significant influence on the mean *M. persicae* count (Table 1).

For the first four days of the experiment, the number of *M. persicae* increased over time (Figure 1). The low dose increased at the highest rate, then the high dose, then the control. At 5 days, there was a small peak in the mean *M. persicae* count for all treatment groups. After 6 days, the mean *M. persicae* count plateaued for the low dose treatment group, while the control and high dose group continued to increase.

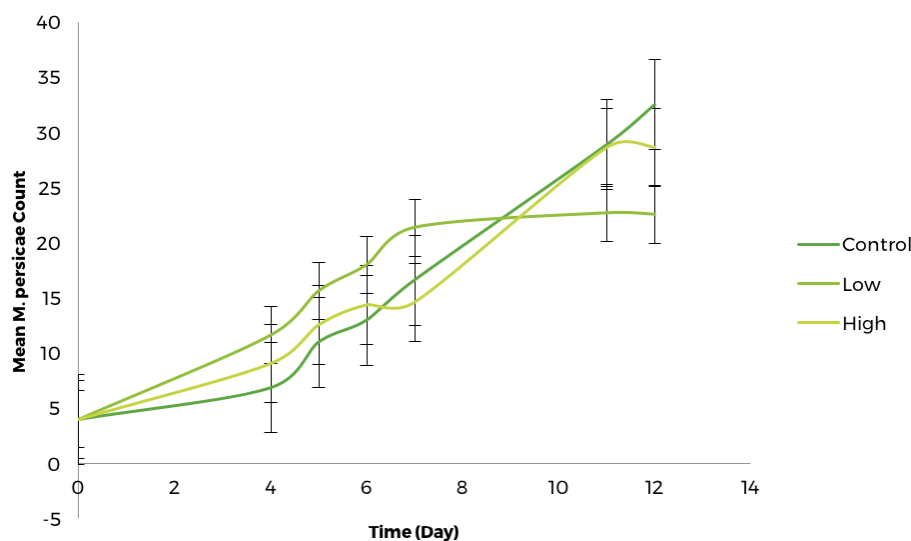


Figure 1: Mean *M. persicae* count over the course of 12 days for plants with no nitrogen added to the soil (Control), plants with 60 ppm ammonium nitrate (AN) added to the soil (Low), and plants with 300 ppm AN added to the soil (High). Error bars represent standard error.

DISCUSSION

Our results suggest that the effectiveness of plant defenses may change based on the concentration of AN added to the soil. Based on our findings, the addition of low doses of AN to the soil of *A. thaliana* can be beneficial when limiting herbivore populations. However, adding higher doses of AN can increase herbivore populations. During the first four days of the study period, the control, low dose, and high dose groups all experienced an increase in mean *M. persicae* count over time (Figure 1). These trends suggest the beginning of an exponential population growth due to back-and-forth competition between the herbivore and plant (Ayala et al., 1973).

On the sixth day, the mean count for the low dose group began to plateau, which demonstrates the potential benefit of a low dose of AN (Figure 1). Uptake of nitrates occurs in the roots of the plant. Here, various transporters move the nitrates from the apoplast of root epidermal and cortical cells, located outside of the plasma membrane of plant cells, into the symplast, located inside the plasma membrane (Sohlenkamp et al., 2002). Nitrogen is an essential nutrient to plants, as it is an important structural component and is essential for metabolic processes, including photosynthesis. By extension, increased levels of nitrogen allow *A. thaliana* to have stronger defenses against herbivory. Our results suggest that the low dose of AN gave *A. thaliana* the extra resources needed to strengthen its defenses, preventing the population of *M. persicae* from growing larger (Kuśnierczyk et al., 2008). Stout, Brovont and Duffey (1998) found a similar result. They reported that low levels of AN addition to the soil produced larger increases in phenolic levels in tomatoes compared to plants with high levels of AN addition. Phenolics are a secondary metabolite that plants release in response to stress. Overall, the plateau in our low dose group rep-

resents the ability of *A. thaliana* to strengthen its defenses against herbivores using AN from the soil.

The benefits of a low dose of AN to the plant also aligns with the theory of resource allocation. All plants must use their resources, including nitrogen, for three main functions: reproduction, defense, and growth, which all interact and compete for resources (Bazzaz et al., 1987). These three functions interact and compete for resources. Allocation to these functions varies by individual and over time, as well as among populations and species (Bazzaz et al., 1987). In the case of our study system, we may see the plateau for the low dose group after the sixth day because the plant has had the chance to react to the herbivory of *M. persicae* and allocate more resources to defense. To confirm this theory, future studies should measure plant biomass and reproductive rates. As demonstrated by Pratt et al. (2005), plants damaged by herbivory will have lower rates of reproduction than undamaged plants. In the case of *M. quinquenervia*, the paperbark tea tree, the undamaged plants were 36 times more likely to reproduce than the plants damaged by herbivory. This points towards resource allocation for defenses, taking away energy from reproduction and growth.

The control group population continued to increase for the duration of the experiment, which suggests the beginning of exponential population growth over our short experimental time period (Figure 1). The high dose group also followed this trend, suggesting that high amounts of AN are not beneficial in promoting the defenses of *A. thaliana* against herbivores. In accordance with this result, Minkenberg and Fredrix (1989) show that leafminers, namely *Liriomyza trifolii*, prefer to feed on plants that were fertilized with higher levels of nitrogen, suggesting that the plant is more attractive to herbivores like *M. persicae*. Other studies have found similar results (Chen, Olson and Ruberson, 2010). In the context of our study system, the increase in attractiveness along with nitrogen concentrations may account for our observed results in the high dose group. Although the *A. thaliana* in the high dose group may have been more attractive to the herbivores, the plateau in the population of *M. persicae* suggests that *A. thaliana* may have exhibited increased defenses. These counteracting forces provide an explanation for the trends observed in our results for the high dose group, which were similar to the control group. Additionally, high levels of nitrogen may have allowed *A. thaliana* to grow larger and thus be able to support more *M. persicae*. However, in our study we did not directly measure plant growth. Further studies should measure growth rate and foliar biomass of *A. thaliana* to determine if increased attractiveness is directly due to increased nitrogen concentrations, or indirectly due to increased

foliar biomass resulting from an increase in nitrogen concentrations.

Despite our promising results, there are several factors that may have altered our data. The *A. thaliana* plants available for this experiment were not of consistent size, age or health, representing a confounding variable. For example, some of the plants entered the bolting stage earlier than others, and based on our observations, there were higher numbers of *M. persicae* on bolting plants than on rosettes. Additionally, since all plants in one treatment group were kept in the same lidded container, there was a possibility of winged alates traveling between plants. Alates may have developed due to lack of space and changes in quality of the host plant (Dixon, 1977). To reduce this error, all plants could have been kept in separate containers. Another source of error is the fact that for the low and high dose groups, we added AN to the soil but did not measure concentrations of nitrogen in the soil itself. These values can be different from one another, as Mulvaney, Khan and Ellsworth (2008) found that addition of AN fertilizer to the soil decreases amounts of natural soil nitrogen. This is because when nitrogen is added to the soil, it can undergo changes in its availability to plants. From this study, we can apply our findings on the effect of nitrogen on aphid population growth to surrounding ecosystem. For example, trophic levels may be affected by nitrogen fertilizers through tri-trophic interactions. This involves the effects of plants, herbivores, and herbivore predators on one another. Nitrogen can affect nutrient availability to plants, which is a bottom-up force that involves the removal of a primary consumer or producer. This bottom-up force can either increase or decrease plant populations (Capinera, 2008). Additionally, this can alter animal community composition, influencing herbivores. This was observed in our study through the interaction between the nitrogen fertilizer treatment and the change in the population of herbivores. Herbivores are also influenced by a top-down force by their predators, carnivores (Capinera, 2008). Carnivores are affected by the populations of their prey, which we know from our study are influenced by the interaction between nitrogen availability for plants, and time (Capinera, 2008). Therefore, all three trophic levels are affected by the addition of nitrogen fertilizer in plants showing the ecological impact of our study and further studies involving nitrogen fertilizers in plants.

Overall, this study provides greater insight into the interactions between plants and animals. Through a controlled laboratory experiment, we found that the addition of ammonium nitrate to the soil of *A. thaliana* has a significant effect on *M. persicae* population growth over time. These findings can be used to better understand

the potential impacts of nitrogen fertilizer runoff on surrounding ecosystems. In addition, our research can be used by future studies as an analogous experiment to apply over a longer time period to analyze the long-term effects of nitrogen on interactions between plants and animals. Further studies must be done to understand all effects of nitrogen fertilizer on the interaction of *A. thaliana* and *M. persicae*, as well as interactions between other plants and herbivores in susceptible environments.

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AUTHOR CONTRIBUTIONS

L.A. contributed to the introduction and discussion. N.K. contributed to the methods and results and performed the statistical analysis. L.B. helped write the introduction, methods, and results and performed the statistical analysis. I.S. wrote the discussion section and the methods for treatment and inoculation. M.S. contributed to the introduction, abstract, and methods sections. All authors contributed equally to experimental design, data collection, and editing.sdfdf

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