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The effect of applying starch onto *Arabidopsis thaliana* on the feeding behaviour of *Myzus persicae*

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ABSTRACT

It is well known that plant-animal systems interact in many complex ways, and each organism must adapt and develop mechanisms to best survive in their given conditions. While much is understood about the plant *Arabidopsis thaliana* and the aphid *Myzus persicae*, additional research must be conducted to gain more knowledge about the interactions between the two species. As a defence mechanism, in response to aphid feeding, *A. thaliana* converts sucrose into starch. Due to a lack of sucrose, there is less feeding by *M. persicae*. However, it has not yet been shown if these aphids are able to detect an increase in starch and recognize this as a deterrent to feeding. To test this, varying concentrations of potato starch were applied mechanically to *A. thaliana* (n=36) and the effect on aphid population size and plant health was analyzed. The research team found that *M. persicae* do not detect higher starch levels on *A. thaliana* as an indicator that nutrient availability on the plant is limited. Instead, it was found that on all but one plant, high starch concentration was a factor in plant deterioration. Thus, the research team advises against using starch as an organic pesticide. The findings of this study are significant as they will contribute to a better understanding of the organisms that threaten plant health, which will prove to be useful in the maintenance of various food crops.

Keywords: *Arabidopsis*, *Myzus*, aphid, starch, feeding, herbivory

INTRODUCTION

Myzus persicae, commonly referred to as the green peach aphid, is one of the most economically important crop pests worldwide[1]. They are generalist herbivores[2] that are globally distributed and have a host range of over 400 different species[1]. In addition to damaging its host plant through feeding, *M. persicae* is a very strong viral vector which can cause the plant to sustain viral damage[3,4]. The main method in controlling *M. persicae* has been using chemical insecticides. The widespread use of these insecticides, aside from being harmful to the environment, has led aphids to develop resistance to many of them[1,5].

When *M. persicae* feeds, it inserts its stylus into the phloem of the plant, effectively wounding it. As a response to the aphid, plants have developed various mechanisms of defense. In *A. thaliana*, one of these mechanisms involves the conversion of sucrose to starch in plant leaves[6,7]. This process is controlled by the enzyme 9 trehalose-6-phosphate synthase 1 (TPS11 9), which promotes the accumulation of starch. *A. thaliana* upregulates the gene that codes for this protein after attack by *M. persicae*[6]. *M. persicae* infestation on *A. thaliana* has also been shown to upregulate the gene responsible for synthesizing ADP-glucose, the sugar that is donated to a growing starch chain. Similar studies have been conducted suggesting that these effects are also seen in tomato plants[2]. In addition, *M. persicae* numbers were found to be lower on plants that were mutant for starch synthase III, which caused starch to be hyper-accumulated. From these findings, it was suggested that starch accumulation in *A. thaliana* deters feeding by *M. persicae*[8,6].

A. thaliana is frequently used in laboratory studies as a model since it is easy to grow and has a fast reproduction cycle[9,10]. For the purpose of this experiment, *A. thaliana* and *M. persicae* are used as a model system to determine how starch applied to the plants will affect the feeding behaviour of *M. persicae*. Since glucose level will not be altered in any of the plants, it is possible to attribute any feeding behaviour changes to the increase in starch levels. Starch will be applied by coating *A. thaliana* with varying concentrations of potato starch. This procedure was chosen since both potato starch and starch produced by *A. thaliana* as a defence mechanism have rounded, discoid granules and appear similar in conformation (although potato starch granules are larger)[9,11,12]. In vivo, potato starch has been found to cause a decreased level of probing from *M. persicae* when compared with a control, also supporting the use of potato starch as a treatment[8].

If the levels of starch on *A. thaliana* have a negative effect on *M. persicae*, it is expected that lower numbers of *M. persicae* will be observed. When under high levels of stress and in environments, *M. persicae* will start to reproduce to create alate morphs. If not under stress, *M. persicae* will be wingless; however, if they are in a stressful environment, their offspring will have wings, allowing for movement to a new location. A complete life cycle of *M. persicae* can occur in 10-12 days, meaning that these changes can be easily and quickly observed[13,14]. If any alate forms are observed on *A. thaliana*, this will also show that *M. persicae* are not receiving enough nutrients, and act as an indicator that the starch has affected their ability to feed.

The null hypothesis for this experiment is: "Different concentrations of starch coatings on the exterior of *A. thaliana* will not affect the feeding behaviour of *M. persicae*." Conversely, the corresponding alternative hypothesis for the research question is: "Covering *A. thaliana* plants in varying concentrations of starch will have an effect on the feeding behaviour of the green peach aphid." The research team predicts that as starch concentration increases, there will be a negative effect on aphid population. The findings of this experiment may contribute to the development of a novel and effective insecticide specific to *M. persicae* that is natural and easy to obtain. This may be used to decrease the usage of commercial pesticides, which are more harmful to the environment and which *M. persicae* have developed a resistance towards[1]. Furthermore, these findings could promote the development of similar insecticides for economically significant crops such as canola, which is also consumed by *M. persicae*[15].

MATERIALS AND METHODS

Obtaining the Study System

A. thaliana seeds that were dried and sealed in envelopes in 2014 were suspended in 1.5 mL of distilled water kept in Eppendorf tubes. Suspensions were placed in the refrigerator to mimic the conditions of springtime, optimizing growth and maintaining natural conditions. Pots were prepared by packing soil and pouring water on top of, as well as underneath the soil, until it was completely soaked. After refrigeration, seeds were placed onto a plate and two seeds were placed in each pot of soil using a micropipette. One was planted in the middle of the pot and the other was planted near the side, as the *A. thaliana* seeds have 70% success rate of establishment. If any of the seeds did not grow, they would be removed from the pot. If two of the seeds succeeded in growing within the same pot, the roots of one plant were dug up and transferred into a pot without any plants. In the end, each pot had one plant. Plants were subjected to 14 hours of light per day for three weeks and then 10 hours of light per day afterwards. Following the third week of growth, fertilizer was applied across the top to prevent damage to the roots. A batch of 500 aphids were ordered from two federal labs. One is the lab of Jean-Philippe Parent, Research Scientist in Entomology, Agriculture and Agri-Food Canada, Government of Canada, Vineland, ON. The other is Agriculture and Agri-Food Canada/Agriculture et Agroalimentaire Canada, Frederic-

Fredericton Research and Development Centre. These aphids were inoculated on *A. thaliana* in domed carriers. In the end, there was a batch stock of 2000-3000 aphids.

Manipulating the Study System

Four stock solutions of Clubhouse potato starch dissolved in water were prepared in four labelled spray bottles. The solutions were divided into a control (0%), low (10%), medium (30%), and high (50%) concentrations of potato starch, created by weighing 0 g, 20 g, 60 g, and 100 g of starch respectively and dissolving each sample in 200 mL of water. *A. thaliana* plants in the rosette phase (n=36) were divided into four groups of nine plants per treatment. For each treatment, plant samples were sprayed five times from a distance of approximately 48 cm from the plant. During this time, the plants were also rotated to ensure that they were entirely covered with the starch solution on both sides of the leaves and on the stem. Solutions were mixed constantly by vigorously shaking the bottle in between sprays to ensure even distributions of the starch.

A stratified random design was achieved by numbering plant samples from 1-36 with plants 1-9 corresponding to the control (red tape), 10-18 corresponding to 30% starch solution (green tape), 19-27 corresponding to 50% starch solution (blue tape), and the 28-36 corresponding to 10% starch solution (yellow tape). Three covered trays contained 12 plants each. Using an online random number generator, a random sequence of numbers from 1-12 were generated. The first three numbers in the sequence corresponded to the positions of the control plants, the next three numbers correspond to low concentrations, the following three numbers corresponded to the medium concentration, and the last three numbers corresponded to the high concentration. Figure 1 is the layout of the position of plants on each tray; three plants were placed per row, with a total of four rows. When placing the plants, care was taken to ensure that they were evenly spaced across the tray so that none of the plants were touching, to reduce the likelihood of aphids transferring between plants within a tray.

Plants were left to dry for an hour before *M. persicae* inoculation. Using a probe, aphids were taken from a variety of plants within a stock tray and two aphids were inoculated on each plant. While inoculating, aphids were placed on top of two leaves on opposite sides of the plant, and aphid colour was recorded.

1	2	3
4	5	6
7	8	9
10	11	12

Figure 1 - Layout of plant position numbers for each of the three trays.

Data Collection

The number of living aphids on each plant, the location of the aphids on the plant, the colour of the aphids, the height of each plant, as well as observations on plant health were then recorded on days one, four, six, eight, and eleven. On each of the data collection days, two researchers counted and measured the aforementioned traits of the same plants. Results were compared to reach a consensus in order to maintain accuracy of any observations made. Days one, four, six, eight and eleven were chosen for collecting data and recording observations. This was to maintain approximately equal spacing between data collection days after inoculation. Between data collection times, trays were covered with a translucent plastic lid to prevent aphids from escaping.

Statistical Methods

A two-way ANOVA and a post-hoc Tukey test were used to analyze the data. The number of aphids counted was transformed on a log(n+1) scale for analysis purposes and analyzed in the software R (R v.3.4.4; The R Foundation for Statistical Computing, 2018).

The differences found between the average number of aphids in 0%, 10%, 30%, and 50% starch concentration levels were measured using a two-way ANOVA, comparing number of aphids with concentration levels, with a covariance analysis on time and an interaction with trays. Testing with a tray interaction would indicate whether or not a block effect is present, despite using a stratified random design. The interaction between starch concentration and time was shown to be insignificant in its effect on aphid number ($F_{15,190}$=0.23, P=0.98), and therefore a three-way ANOVA was not used. To gain a better understanding of our data, a post-hoc Tukey test was conducted to determine which level(s) in our treatment groups of starch concentration and trays caused significant differences between the means of *M. persicae* population size.

RESULTS

QUALITATIVE ANALYSIS

Trends between the varying concentrations of starch

Plants with solutions of 0% and 10% starch concentration mainly experienced a decrease, then increase in aphid number, with a few cases of disappearance. Plants with solutions of 30% starch concentration also experienced a similar trend of decrease, followed by an increase in aphid number. Finally, plants with solution of 50% starch concentration primarily demonstrated cases of aphid disappearance.

Plants 4 and 21 from the 0% and 50% starch concentration solutions were initially inoculated with red aphids. However, throughout the 11-day period red aphids appeared on two different plants with 0% starch concentration solution and on one plant with 10%, 30% and 50% starch concentration solutions each (excluding plants 4 and 21). Most plants had aphids that were initially found under leaves, which eventually relocated to the plant stems. In addition, a majority of plants from all treatment groups had yellowing leaves. On plants sprayed with 30% and 50% starch concentration solutions, there was greater deterioration and moulding of leaves. In terms of plant height, all four treatment groups experienced an initial growth period, which was then followed by a subsequent decrease.

Trends between the trays

The first, second and third trays experienced 17%, 50%, and 42% of cases of aphid disappearance respectively. The first and third trays only contained one plant with red aphids, whereas the second tray contained three plants, each with their own population of aphids. Overall, most aphids progressed from being on leaves to stems. The second and third trays' plant samples experienced yellowing of their leaves, but also exhibited budding. Conversely, the first tray's plant samples demonstrated an equal spread in terms of overall plant health. An initial increase followed by a decrease in plant height was prominent in all trays. Furthermore, the disappearance of aphids and plant health was similar in the second and third trays.

QUANTITATIVE ANALYSIS

In terms of aphid population, time ($F_{5,199}$=5.5386, P<0.001), starch concentration ($F_{3,199}$=3.4180, P<0.05), and trays ($F_{2,199}$= 9.7554, P<0.001) were all significant independent variables. A two-way analysis of variance was performed and there was a significant effect of starch concentration and tray on aphid population ($F_{6,199}$=2.1920, P<0.05). The post-hoc Tukey HSD test shows the specific differences as described below.

For time, there were significant differences in overall aphid population between days 11 and 1 (P<0.001), days 11 and 4 (P<0.01), and days 11 and 6 (P<0.05). Whether the data was qualitatively compared by categorizing concentrations or by trays, the general trend was a decrease in population from day 0 to day 1 and then an overall increase in aphid number by day 11 (Figure 2).

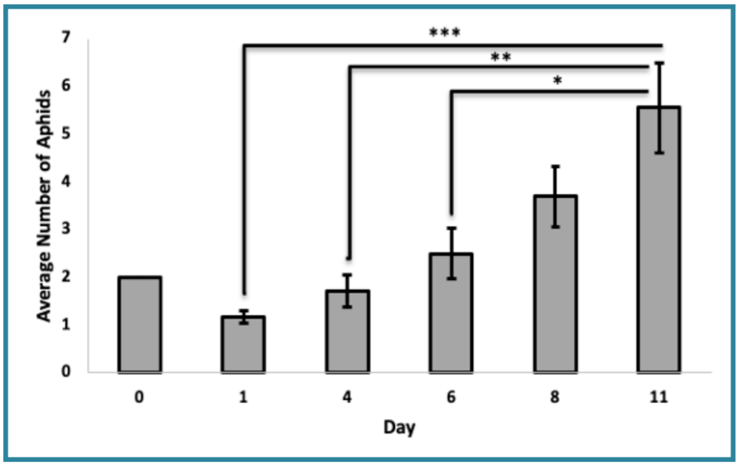

Figure 2 - Average number of aphids on each day of data collection. Error bars represent the standard error of the mean (SE). SE= ± 0, 0.129099445, 0.341823103, 0.523268118, 0.635983733, 0.940655331 for days 0, 1, 4, 6, 8, and 11, respectively. There were significant differences in aphid numbers between days 11 and 1 (***P<0.001), days 11 and 4 (**P<0.01), and days 11 and 6 (*P<0.05). After the decrease in population from days 0 to 1, there was a steady increase in aphid number until day 11.

The significant differences in aphid population due to concentration were between the 50% and 10% (P<0.05) as well as the 50% and 30% (P<0.05) starch concentration solutions (Figure 3).

There was a significant difference (P<0.001) in aphid population between the second and first trays, as well as the third and first trays. This was observed qualitatively for cases involving aphid disappearance in both the second and third trays (50% and 42%, respectively) and for the first tray (17%). In addition, overall plant health remained relatively consistent, and appeared similar between the second and third trays. On the other hand, a wider range of overall plant health was seen in the first tray samples (Figure 4).

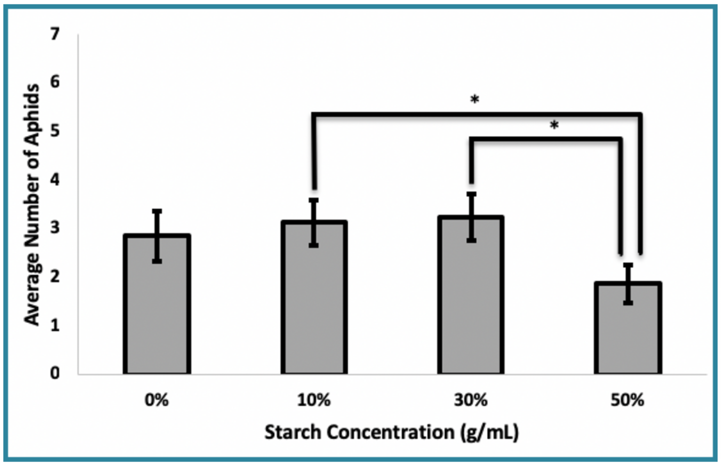

Figure 3 - Average number of aphids for each treatment of starch concentration (g/mL). Error bars represent standard error of the mean (SE). SE= ± 0.526321443, 0.469210122, 0.48667441, 0.392136452 for concentrations 0%, 10%, 30%, and 50%, respectively. There was a significant difference (*P<0.05) in aphid population between starch concentrations of 50% and 10% as well as the 50% and 30%. A significant decrease in aphid number was observed following treatment of the 50% starch concentration.

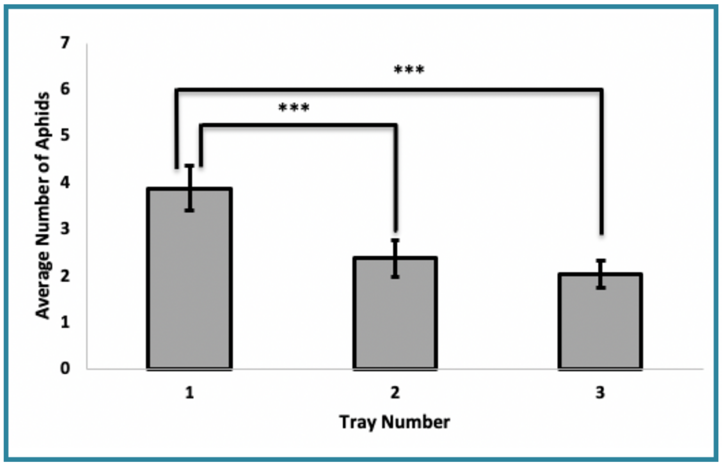

Figure 4 - Average number of aphids on each tray. Error bars represent standard error of the mean (SE). SE= ± 0.489944088, 0.390895783, 0.298953247 for tray numbers 1, 2, and 3 respectively. There was a significant difference (***P<0.001) in aphid population between the second and first trays and the third and first trays. There were significantly more aphids on the first tray than the second and third tray.

Out of all of the samples observed, the most significant differences of aphid population were seen for the 10% and 30% starch solutions in the first tray. There were also significant differences between the 30% and 50% concentrations in the third tray, as well as the 10% concentration in the first tray (P<0.05; P<0.001), along with a difference between the 50% concentration in the third tray and 30% concentration in the first tray (P<0.01). Lastly, there were significant differences between the controls in the second tray and 10% and 30% concentrations of starch in the first tray (P<0.01; P<0.05).

DISCUSSION

The goal of this study was to determine whether *M. persicae* would detect high levels of starch concentrations on a plant and thus be deterred from feeding on it. Statistical analysis and observations of the collected data showed that differing levels of starch have an effect on *M. persicae* abundance. From this experiment's results, there was no strong indication that the starch directly deterred the aphids from feeding on the plants. Alternatively, the *A. thaliana* plants treated with 10% and 30% starch concentrations unexpectedly showed higher numbers of *M. persicae* than the control plants. These results suggest that *M. persicae* do not interpret increased levels of starch on a plant as a signal of it having limited nutrients. It is also possible that *M. persicae* simply cannot detect increased levels of starch on a plant.

Based on qualitative observations, it is worth noting that plant health was visibly worse on the samples treated with 30% and 50% starch solutions. A possible explanation for this is that the starch solution- which dried as clumps- blocked the plants from sunlight, thus inhibiting photosynthesis and stunting their growth. Based on these observations, the samples of *A. thaliana* coated with 50% starch solution showed a general decrease both in the *M. persicae* population size, and in plant health. This led us to believe that these negative effects were likely not a cause of *M. persicae* feeding, but rather a consequence of blocked sunlight. Furthermore, the control and 10% starch solution groups showed increases in *M. persicae* population size near the end of the observation period. However, the extent of plant health deterioration was not as great in these two samples, as was observed for the 30% and 50% starch treatment groups.

It is important to recognize that feeding by *M. persicae* on *A. thaliana* did still play a role in deteriorating plant health. Some signs of this in the samples that showed high levels of feeding are i) plant leaf yellowing and curling from losing moisture or by toxins that may have been injected by *M. persicae* and ii) blackened plant mass and growth of sooty mold fungi, which originate from "honeydew", a sticky substance produced by *M. persicae* during feeding[16]. The former was seen in all samples except for plant number 10, which was in the control treatment. As such, there does not seem to be a strong relationship between starch concentrations applied onto the plant, and the amount of feeding by *M. persicae*. Interestingly, in the case of the latter, most moldy plants observed were found to be under the 30% and 50% starch solution treatment. Since no definitive relationship can be determined from such information, it is proposed that the blockage of sunlight by the opaque clumps of starch likely contributed to the plant deterioration seen in the 30% and 50% starch treatment samples.

Looking at the data, a trend can be seen whereby a higher number of *M. persicae* existed on the 10% and 30% plants than on the 50% plant and the control. While it was predicted that higher starch concentrations would decrease the numbers of *M. persicae* feeding on *A. thaliana*, the opposite occurred. That is, samples with higher starch concentrations actually increased aphid population for the intermediate treatments, compared to the control. If it is true that the starch negatively impacted *A. thaliana*, then it is possible that the carbohydrate reduced the plant's defense capabilities, which would explain the increase in *M. persicae* numbers seen on the 10% and 30% starch concentration plants. The 50% concentration may have negatively affected the plant to a degree that caused it to have lower levels of sucrose, thus reducing the food available for *M. persicae* and causing a subsequent decrease in the aphid population.

Some possible limitations of this study include the sample size, tray locations, and ability to apply starch uniformly across the entire plant. In terms of sample size, only nine plants were studied per treatment group. In the future, the design of this experiment may be improved upon by increasing sample sizes, to better control for any variance between individual samples that may have impacted the number of aphids that were seen on each plant. These variations include differences in leaf surface area and number of leaves on each plant. Pertaining to tray location, the trays were placed under a window to allow for the exposure to sunlight, which was necessary for growth. However, due to the limited size of the window, it is possible that all trays did not receive equal or optimal sunlight, which may have contributed to plant deterioration. Lastly, applying the starch via a spray bottle allowed for consistent spraying of all plants. However, the starch dried in droplets, meaning that the exterior of the plant was not covered evenly at all times. This could be ameliorated by using different methods of starch application, such as using a brush, as well as periodically spreading the starch overtime. This could be performed to ensure that the entire plant is thoroughly and evenly covered at all times.

CONCLUSION

From these observations, the research team rejects the null hypothesis. Covering *A. thaliana* plants in varying concentrations of starch did appear to have an effect on aphid feeding behaviour, as depicted by the significant differences between aphid number in 50%-10% and 50%-30% starch concentration. However, since there was no clear trend between increased starch concentration and aphid numbers on *A. thaliana*, these findings show that the motivation of creating a less environmentally harmful, starch-based pesticide would not be successful. Nonetheless, the results from the study indicate that there was a significant difference in the aphid population between differing starch concentrations, and that potato starch may have an effect on *A. thaliana* health. With more investigation into the potential of starch as a natural pesticide, this compound may prove to be a useful resource in reducing crop damage. Similar principles can be applied to more economically important plants, such as canola, in order to develop insecticides that would deter aphids, and trigger plant defences in a similar way.

Further studies should be performed to corroborate the findings by the research team. As such, the team would suggest replicating this experiment over a longer period of time, with a higher sample size and more treatment groups. Additionally, future researchers could mechanically apply the potato starch to the plants, so that the starch is spread out. This will help to avoid clumping, and the subsequent blockage of photosynthesis.

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