Effects of anthropogenic acetylsalicylic acid contamination on ecological interactions

ANGELA DITTRICH, JOSEPHINE AGUECI, TAYLOR LUU, MEGH RATHOD, NADIA AL HASHEMI
Integrated Science Program, Class of 2020, McMaster University

SUMMARY

Pharmaceutical soil contamination has become a rising concern based on its potential impacts on ecological interactions. Acetylsalicylic acid (ASA), also known as Aspirin, is a commonly used medication that has been found to have an impact on plant defence by enhancing salicylic acid immunity pathways. This study aimed to determine the impact of varying ASA soil concentrations on the population growth of Myzus persicae on genotypically different strains of Arabidopsis thaliana. Additionally, this study analyzed how varying ASA soil concentrations impact the plant growth of A. thaliana. Two ascension lines of A. thaliana were used in this experiment: the wild-type Col line and the mutant salicylic acid induction-deficient sid2. Each plant was treated with one of four aqueous ASA concentrations (0M, 1.1nM, 11nM, 0.2mM). Overall, sid2 plants experienced a negative mean aphid population growth rate relative to the Col plants. It was concluded that neither genotype nor ASA concentration had a significant impact on the population growth of M. persicae. Interactions between genotype and ASA concentrations were found to be significant for both aphid population and bolt height responses. A greater reduction in aphid population was observed as ASA concentration was increased for both genotypes, while an inverse relationship was observed between ASA concentration and bolt height growth. There were no observable effects of bolt height on M. persicae population growth. Overall, investigating the interactions between M. persicae and A. thaliana in the presence of acetylsalicylic acid provides further evidence for the ecological impact of pharmaceuticals, and the importance of minimizing this contamination.

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INTRODUCTION

For centuries, humans have been altering their surrounding environment in detrimental, and often irreversible, ways. The anthropogenic contributions to climate change have directly and indirectly impacted ecosystems by altering pH levels, temperature, climate, humidity, salinity, and nutrient availability in soil and water. One major concern that has become prevalent in the last decade is the introduction of pharmaceuticals to the natural environment (Association of Metropolitan Water Agencies, 2008). It is estimated that pharmaceutical use continues to rise 10 to 15 percent annually in North America (Health Canada, 2012). This increase, combined with over-prescription and improper disposal of medication, results in increasing chemical contamination of lakes and rivers via direct runoff or through human excretion (Crowe, 2014). While a wide variety of studies have observed and analyzed the effects of antidepressants (Metcalfe, Miao, Koenig and Steuger, 2003; Schultz et al., 2010; Lajeunesse et al., 2012) and contraceptives on the endocrine systems

of aquatic organisms such as fish and frogs (Park and Kidd, 2005; Kloas et al., 2009; Runnalis et al., 2013; Orlando and Ellestad, 2014), the impacts of pharmaceutical absorption by plants is severely understudied. Many plants rely on inducible chemical pathways as a defence mechanism, so altering the presence or concentration of various compounds even slightly could have major consequences (War et al., 2012). Thus, it is crucial that the effects of common medications on ecological interactions is examined to quantify the magnitude of these consequences. An investigation was conducted on the impacts of soil contaminated with acetylsalicylic acid on plant-animal interactions. Commonly known by the brand name Aspirin, this nonsteroidal anti-inflammatory drug is a derivative of salicylic acid (SA), a signalling molecule that plays an important role in the plant immune system via SA-inducible defence pathways (Clissold, 1996). Salicylic acid acts as a stress messenger and has been linked to resistance against biotrophic pathogens and promotion of Systemic Acquired Resistance (SAR), which 'primes' plants following an attack, ensuring faster and more effective defence responses against a second attack. (Conrath, 2006, Morkunas; Mai and Gabrys, 2011). Another major plant defence is the jasmonic acid (JA)-inducible pathways, which also involve the use of JA molecules as messengers that activate certain genes to address predation. However, unlike the SA pathways, JA pathways have been observed to be an effective response to stressors such as necrotrophic pathogens and herbivorous insects (Conrath, 2006). The presence and activation of both pathways within an individual plant results in crosstalk in an attempt to optimize resource allocation and employ the most effective defence response respective to the predator. However, it is evident in the following studies that it has been observed that SA and JA pathways share an antagonistic relationship when confronted by certain biotic stresses. Although JA-induced mechanisms are more effective against predators such as generalist aphids, the SA signalling pathway has been observed to suppress the JA pathway by means that are still unclear. Leon-Reyes et al. (2010) concluded that SA-mediated suppression of the JA signalling pathway targeted regions downstream of the JA biosynthesis pathway. Observations have also been provided towards SA-mediated suppression of JA-inducible genes (Kourneef et al., 2008). Nevertheless, suppression of the JA signalling pathway has provided evidence for the secretion of SA-inducible compounds by green peach aphids such as *Myzus persicae*, in order to upregulate the less effective plant defence mechanism (Louis and Shah, 2013).

Interest in crosstalk between the SA and JA signaling pathways has led to the experimental use of ascension lines deficient in one of the mechanisms (Nishimura and Dangl, 2011). In the present investigation, the Columbia (wild-type) ascension possesses functional SA and JA pathways, while the *sid2* ascension possesses a mutation to the SA-induction deficient 2 gene in *Arabidopsis thaliana*. This gene codes for isochorismate synthase, an enzyme which plays a crucial role in SA biosynthesis (Ghazijahani et al., 2014). Mutation of this gene results in plants with a reduced ability to accumulate SA, and thus enhanced susceptibility to pathogens and environmental stresses. Salicylic acid (SA) is one of the most readily available plant growth regulating materials, and is also effective in other forms such as methyl salicylate (Ghazijahani et al., 2014).

The objective of this study was to address the following two questions: (1) How do varying ASA soil concentrations impact the population growth of *M. persicae* on *A. thaliana* across different plant genotypes? (2) How do varying ASA soil concentrations impact the growth and overall quality of *A. thaliana*? The corresponding null hypotheses were the following: (1) ASA concentration and genotype do not affect aphid population. (2) ASA concentration does not impact the growth and overall quality of *A. thaliana.*

MATERIALS AND METHODS

This experiment was conducted over a span of 12 days in an indoor facility at McMaster University in Hamilton, Ontario, from September 21st to October 3rd, 2017. All 32 *A. thaliana* plants (16 *Col* and 16 *sid2*), were in rosette or early bolting stage. The average lifespan of *A. thaliana* is 60 days (Serino and Gusmaroli, 2011), so selecting plants in these stages minimized flowering and plant death prior to the completion of the experiment. Plants were divided into two separate groups based on ascension line (*Col* or *sid2*), with four different ASA treatments within each ascension line (0M, 1.1nM, 11nM, 0.2mM). The justification for the four concentrations were: 0M was the control to observe response variables without exposure to ASA, 1.1nM and 11nM were the lower and upper bounds of ASA concentrations that have been recorded in the environment (Cleuvers, 2004), and 0.2mM was a physiologically active concentration observed to induce plant immune responses (Senaratna et al., 2000). A double tray apparatus was used to minimize cross contamination of different water treatments between neighbouring plants. The trays were structured with a solid plastic tray on the bottom, and a permeable tray layered overtop to allow for drainage of water and the solute it may contain. A gap between the trays was created by folding paper towels and wedging them along the perimeter of the two trays creating separation. Each tray corner was labelled, creating a standardized grid system to define the location of each plant and the varying orientations of the trays

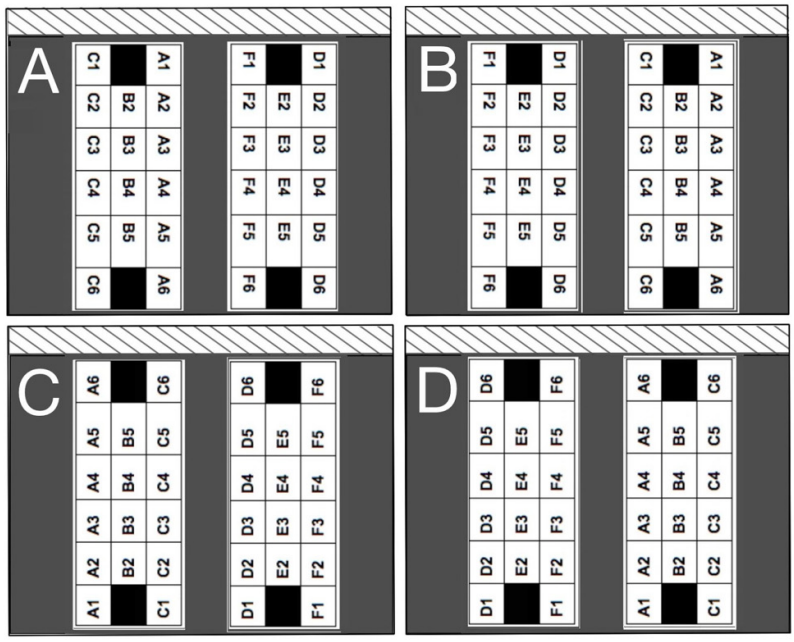

Figure 1: Initial experimental setup. The *Col* tray contained plants A1- C6, while the *sid2* tray contained plants D1- F6. The initial setup on Day 0 (A) had the *Col* tray to the west of the *sid2* tray, with the "1" side of the tray against the north window ledge as illustrated by the striped area. The trays were switched on Day 5 (B) with the "1" side of the tray against the north window ledge but the *sid2* tray to the west of the *Col* tray. The trays were switched on Day 5 (B) with the "1" side of the tray against the north window ledge but the *sid2* tray was placed to the west of the *Col* tray. On day 8 (C), the "6" side of the tray was placed against the north window ledge with the *Col* tray to the west of the *sid2* tray. On day 11 (D), the "6" side of the tray was placed against the north window ledge with the *sid2* tray to the west of the *Col* tray.

throughout the experiment. Trays were re-oriented on days 5, 8, and 11, to eliminate any environmental bias (see Figure 1). A number generating software (Haahr, 1998) was implemented to randomly assign a location and ASA concentration to each plant.

PREPARING TREATMENT SOLUTIONS AND INOCULATION WITH APHIDS

The ASA solutions were prepared from 100 g of 99% powdered acetylsalicylic acid and distilled water on a VMR stirrer. 10 drops of MeOH were added to ensure solubility in water. 15 millilitres of the corresponding SA solutions were exogenously delivered to the plant via soil drenching. The plants were first watered on Day 0 with respective solutions and twice more throughout the duration of the experiment (Day 4 and Day 11). On Day 4, three aphids were inoculated from pre-existing *A. thaliana* plants onto the rosette leaves of each of the 32 test plants. The aphids were all in an intermediate development instar stage and were reared on a wild type (*Col*) *Arabidopsis thaliana*. Rearing them on *A. thaliana* ensures the aphids were not starved prior to inoculation, avoiding any malnourishment. As well, during the investigation, the aphids were subjected to the same conditions by maintaining spatial and temporal proximity of the population. Following the inoculation, aphid numbers were counted for the duration of the experiment by inspection with a probe and magnifying glass. Bolt height was measured on days 0, 4, 8, 11, and 12, as a quantitative factor coupled with qualitative observations, serving as a proxy for plant quality.

STATISTICAL ANALYSIS

The effects of genotype and ASA concentration on aphid population growth were assessed with an ANCOVA, followed by a post hoc Holm's test. Time, measured in days, was interpreted as a continuous independent variable. The effects of genotype and ASA concentration on bolt height growth were also assessed with an ANCOVA followed by Holm's test. Possible block effects arising from growth of *Col* and *sid2* plants in different trays were included in the above analyses. The potential impacts of bolt height on aphid population growth were assessed using a linear regression model. All analyses were carried out using R version 3.3.2 (R Development Core Team, 2016).

RESULTS

APHID POPULATION GROWTH

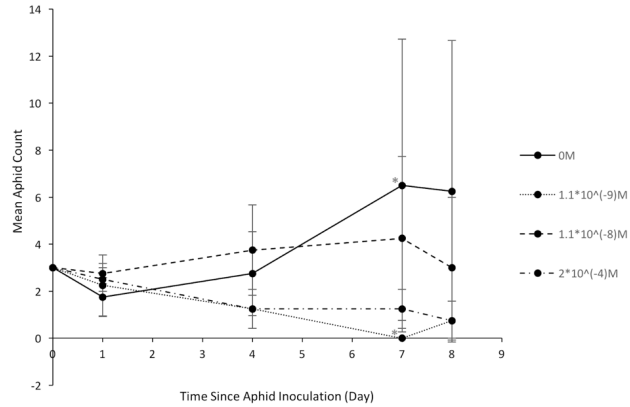

Figure 2: Mean Aphid Count of *Col* for Four Concentrations over Eight Day Period. Control = 0M, Low = 1.1nM, Intermediate = 11nM, High = 0.2mM. Error bars show standard deviation (<0.95 interval).

Each *A. thaliana* plant used in this experiment was exposed to one of four ASA concentrations: 0M (control), 1.1nM (low), 11nM (intermediate) or 0.2mM (high). Among all eight combinations of genotype and treat-

Table 1: Three-Way Analysis of Covariance of the Effect of Genotype and ASA Concentration Difference on *Myzus persicae* Population Growth Across Eight Day Test Period. * indicates a significant p-value.

Source	d.f.	Mean Square	F Value	Pr(>F)
Plant x Treatment x Day	3	20.7156	4.7946	0.003255*
Treatment x Day	3	13.2290	3.0618	0.030168*
Plant x Day	1	12.4256	2.8759	0.092078
Plant x Treatment	3	23.7750	5.5026	0.001318*
Day	1	8.2656	1.9130	0.168766
Treatment	3	16.0417	3.7128	0.013064*
Plant	1	30.6250	7.0880	0.008643*
Residuals	144	4.3207		

ment, the control *Col* plants experienced the only net increase in mean aphid count over the test period, peaking at a mean of 6.50±6.22 aphids on Day 11 (Figure 2). This peak is significantly different (P<0.05) from only two other treatment combinations (low *Col* on Day 11, d.f. = 3, p = 0.039; and intermediate *sid2* on Day 12, d.f. = 3, p = 0.039) (Table 1). These were the only significant differences found with interaction between genotype, treatment, and day. The high *sid2* plants also had a similar trend, exhibiting the largest increase among all *sid2* treatments and peaking at 3.50±2.29 aphids on Day 11 (Figure 3). The high and low *Col* plants exhibited a gradual decrease in mean aphid count, while the intermediate *Col* plants gradually increased over the test period, but had no overall change. However, for *sid2*, mean aphid count decreased from Day 4 to Day 12 for all concentrations without a significant difference between one another, suggesting that they were similarly negative during this time period.

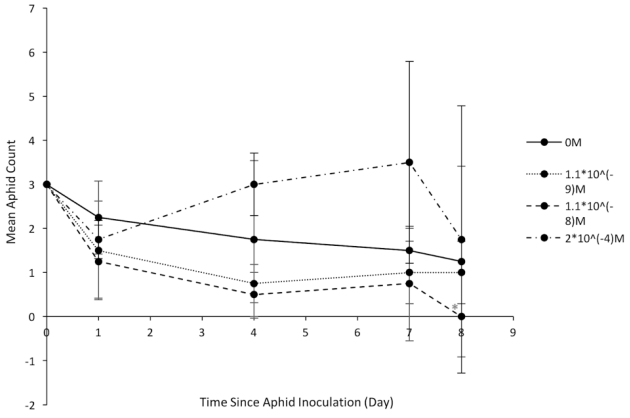

Figure 3: Mean aphid count of *sid2* for four ASA concentrations of eight day period. Error bars show standard deviation (<0.95 interval).

Additionally, observations were made regarding overall change in aphid count from Day 4 to Day 12 (Figure 4). The control *Col* experienced the only increase observed among all treatments and genotypes with a mean increase of 3.25±6.42 aphids, approximately double that of the *sid2* decrease. The low concentration group ex-

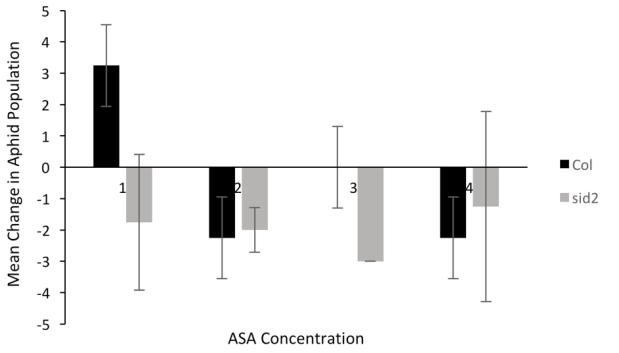

Figure 4: Mean Change in Aphid Count of *Col* and *sid2* Plants at Various ASA Levels over Eight Day Test Period. '1', '2', '3', and '4' represent 0M, 1.1nM, 11nM and 0.2mM. Error bars show standard deviation (<0.95 interval).

pressed a similar overall decrease in mean aphid count in both the *Col* and *sid2* plants of -2.25±0.83 and -2±0.71, respectively. The intermediate concentration group exhibited 0±3.00 in mean aphid population for *Col* plants and a -3±0.00 change in aphid population count in the *sid2* plants, the largest decrease observed among the genotypes and treatments. The high *Col* and high *sid2* plants expressed a change in aphid count of -2.25±0.83 and -1.25±3.03, respectively. No significant differences were found in the overall change in mean aphid count between genotypes for any treatment (d.f. = 3, p = 1.000 for almost all of differences in mean) (Table 2).

Table 2: Significant interactions (P < 0.05) between genotype and ASA concentration on aphid population count across eight day period since ASA inoculation

Genotype. Conc.Day	Sid. high.8	Col. control.11	Sid. control.11	Col. low.11	Sid. low.11	Col. int.11
Col.low.11	1.000	0.039	1.000	-	-	-
Sid.int.12	1.000	0.039	1.000	1.000	1.000	1.000

BOLT HEIGHT

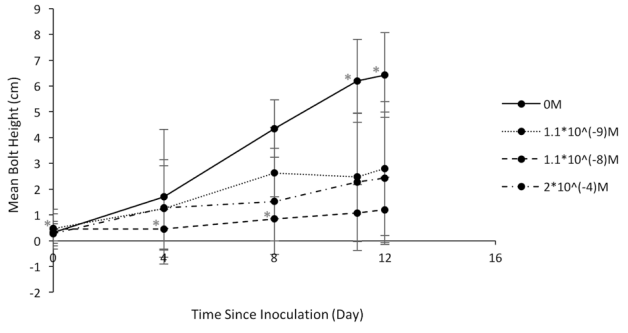

Figure 5: Mean bolt height of *Col* plants for four treatments for 12 days since initial inoculation with ASA. T0 = 0M; T1 = $1.1x10^{-9}$M; T2 = $1.1x10^{-8}$M; T3 = $2.0x10^{-4}$M. Error bars show standard deviation (<0.95 interval).

Interactive effects between genotype and concentration on mean bolt heights of *Col* (Figure 5) and *sid2* (Figure 6) plants were observed on specific days over the 12-day test period (Figure 7). All *Col* ASA concentra-

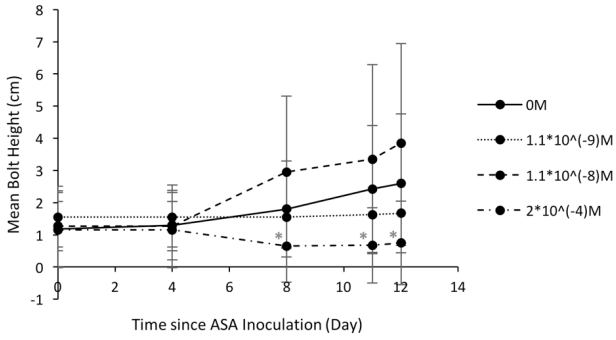

Figure 6: Mean bolt height of *sid2* plants for 4 treatments over 12 days from initial ASA inoculation. Error bars show standard deviation (<0.95 interval).

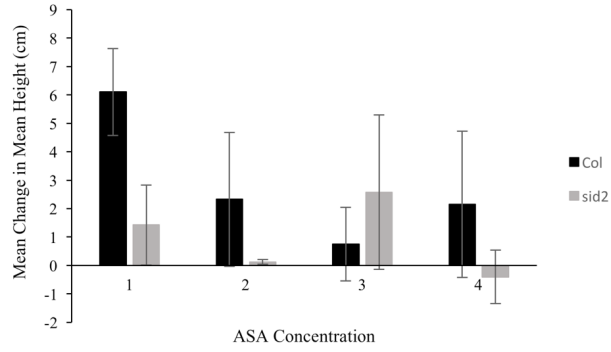

Figure 7: Mean change in plant bolt height of *col* and *sid2* plants at various ASA levels over 12-day test period. '1', '2', '3', and '4' represent 0M, 1.1nm, 11nm and 2.0µm. Error bars show standard deviation (<0.95 interval).

tions exhibited positive bolt height growth, while *sid2* exhibited varying trends across different ASA concentrations. Control *Col* plants demonstrated the greatest mean bolt height growth compared to the three other ASA concentrations, with a steady increase ending at 6.43±1.64cm. Bolt height means on Day 11 and Day 12 for control *Col* were significantly different from Day 0 means for all treatment groups (Table 3; d.f. = 3, p = 0.0109, p = 0.0094, p = 0.0159, and p = 0.0170, from control to high respectively). Control *Col* on Day 12 was also significantly different compared to high *sid2* on Day 8 (P<0.05, d.f. = 3, p = 0.0283) and Day 11 (d.f. = 3, p = 0.0304). Bolt height growth for low and high *Col* were similar in both progression and final heights of 2.8±2.60cm and 2.43±2.56cm, respectively, while intermediate *Col* demonstrated the smallest bolt height growth (1.20±1.27cm). In addition, *Col* exhibits greater bolt growth at all ASA concentrations compared to *sid2*, except at intermediate concentration, where *sid2* mean growth is approximately three times greater. At high ASA concentration, the only overall decrease in bolt height is observed in *sid2* plants, while *Col* bolt growth is similar to low ASA concentration.

The control and intermediate *sid2* plants demonstrated increasing bolt height growth rates, with that of the intermediate plants being at a steeper incline. Almost no change in mean bolt height was observed at low *sid2*. The notably small standard deviation (0.125±0.083cm) suggests the presence of a threshold of effect of ASA concentration on bolt height. Interestingly, all *sid2* ASA concentrations had very similar growth rates from Day 0 and Day 4, after which the different treatments diverged with rates that were not significantly different (d.f. = 3, p = 1.0000). Most importantly, bolt height growth appeared to share an inverse relationship with ASA concentration among both *Col* and *sid2* plants.

HEIGHT ON APHID POPULATION GROWTH

The intercept of the linear regression plot of bolt height against aphid population was significant (Table 4; p = 5.82e-15) while the slope was not (d.f. = 1, p = 0.828) (Figure 8).

Table 4: Linear regression model of the effect of height on aphid population for all genotypes and treatments. * indicates a significant p-value.

Source	d.f.	Estimate	Pr(>|t|)	F	Adj. R^2
Intercept		2.07448	5.82e-15*	0.04741	-0.007679
Height	1	-0.01722	0.828		
Residuals	124				

DISCUSSION

APHID POPULATION

Control *Col* exhibited the greatest overall increase in *M. persicae* populations, peaking on Day 11 (Figure 2 and Figure 3). This was contrary to the expectation that control *Col* would experience the greatest decrease in aphid population compared to all other *Col* treatments, as the plants had the smallest degree of crosstalk between SA and JA, due to the absence of upregulation of SA by ASA. Evidence from other studies suggests that SA-inducible pathways do not play an important role in addressing aphid feeding stress. For example, mutant *A. thaliana* such as *sid2* and npr1, which have a loss of key SA regulator and receptor NPR1 (non-expressor of PR genes 1), have unchanged aphid population growth compared to wild-type (Moran and Thompson, 2001;

Table 3: Significant interactions (p < 0.05) between genotype and ASA concentration on bolt height across eight-day period

Genotype. Conc.Day	Col. cont.0	Col. high.0	Col. mod.0	Col. low.0	Col. mod.4	Sid. high.8	Col. mod.8	Sid. high.11	Sid. high.12
Col.control.11	0.0212	0.0183	0.0304	0.0325	0.0304	-	-	-	-
Col.control.12	0.0109	0.0094	0.0159	0.0170	0.0159	0.0283	0.0498	0.0304	0.0375

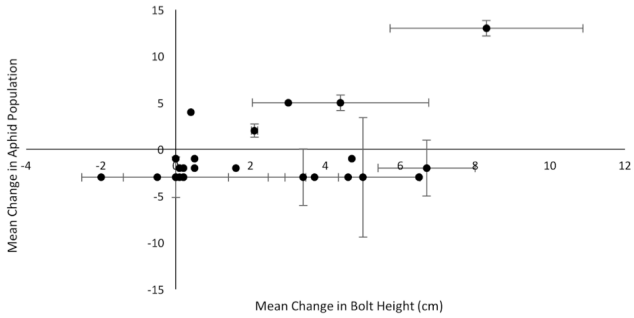

Figure 8: Relationship between mean change in bolt height and mean change in aphid population across all genotypes and ASA concentrations

ticeable increase in aphid population numbers on control *Col* conflict with the above evidence. The absence of ASA in control *Col* should favour SA pathways to the smallest degree among all *Col* treatments and thus result in reduced aphid populations. Although significant differences in aphid population count were only found between control *Col* on Day 11, and low *Col* and intermediate *sid2* on Day 11 and Day 12 (d.f. = 3, p = 0.039; d.f. = 3, p = 0.039), evidence for interaction between genotype and ASA concentration demonstrated by the trend of control *Col* may suggest a greater degree of effectiveness of SA-inducible pathways against aphid feeding than previously believed. Conflicting observations have been made on the exogenous application of SA derivatives on aphid settling and growth; application of synthetic analogs of SA has no impact (Moran and Thompson, 2001), while the SA analog benzothiadiazole has resulted in decreases in aphid reproduction (Morkunas, Mai, and Gabriś, 2011). Furthermore, several SA-related pathways have been identified as effective defences against aphid feeding. For example, the regulatory gene PAD4 (phytoalexin deficient4) is one of the genes responsible for inducing SA synthesis, as well as activating a defence pathway that adversely impacts aphid settling and fertility (Wiermer, Feys, and Barker, 2005). In turn, SA molecules enhance PAD4 expression, resulting in a positive feedback loop. Thus, it is suggested that SA-inducible pathways, while possibly less effective than counterpart JA pathways, may still play an important role in reducing aphid population numbers. This is supported by the low, intermediate and high treatments of both genotypes - excluding high *sid2*, which exhibited only decreases in aphid population - which suggest a more effective plant defence response to aphid feeding with greater exposure to ASA (Figure 3). The lack of significant differences between *Col* and *sid2* suggests that they were similarly effective in plant defence, and that exogenous exposure versus no exposure to ASA impacts plant response more greatly than degrees of ASA concentration. This may also suggest that addition of ASA via soil drenching may be an effective substitute for SA in the absence of endogenous SA.

Support for a greater role for SA-inducible pathways is contradicted by observations made with high *sid2*. Exposure to the highest ASA concentration in *sid2* was expected to yield the highest aphid population numbers among all *sid2* plants due to greatest suppression of JA. However, the small mean aphid population on high *sid2* might be explainable by error. From Day 11 to Day 12, all genotype and treatment combinations experienced a decrease in aphid population, excluding a small increase observed among low *Col* that is likely a consequence of aphid migration. This shared decrease may be due to contamination of the ASA solutions produced on Day 0 and used throughout the experiment for soil drenching. A contaminant must have been introduced, as the development of an unidentified biological contaminant was observed in the high ASA concentration beaker on Day 11, thus contamination of the other solutions may have been unnoticeable to the naked eye. The contaminant was observed to be cloudy yellow-white in colour, irregular in shape, and filamentous (refer to Figure 9). This common decrease may have also arisen from the introduction of predators into the environment. Aphid predators were first noticed on Day 8, and could have potentially been responsible for any drops in aphid count observed throughout the experiment. Thus, had the experiment ended on Day 11 rather than 12 due to significant impact by error, the trend of mean aphid count on high *sid2* would have become an overall increase, suggesting that upregulation of SA-inducible pathways adversely impacts plant defence. However, this appears to be an outlier trend and may suggest the presence of a threshold ASA concentration at which ASA exposure changes from positively to negatively impacting plant defence.

Figure 9: Unknown contamination observed in the high ASA concentration beaker on Day 11.

Consideration of more ASA concentrations within the experimental setup of a future study may make these trends more discernable, and provide more accurate observations regarding the importance of the SA signalling pathway. This would be aided by the inclusion of an ascension line possessing a non-functioning JA signalling pathway, in order to further investigate the impacts of JA-SA crosstalk.

Interestingly, mean aphid population decreased on all plants between the first and second day since aphid inoculation, irrespective of genotype or ASA concentration. This was believed to arise from stress placed on the aphids as they were introduced to a new environment and each of the plants were watered with the appropriate ASA solution.

BOLT HEIGHT

There is an apparent inverse relationship between ASA concentration and change in bolt height for both genotypes, supported by all treatment combinations except intermediate *Col* and *sid2*. At higher ASA concentrations, reduced bolt height growth was observed. While significant differences were only found between control *Col* on Day 11 and Day 12, and the other three ASA concentrations of *Col* and high *sid2* (Table 4), this trend was expected. SA plays an integral role in plant senescence and cell death, and exogenous application of SA causes programmed cell death (PCD) and early senescence in association with SAR (Vogelmann et al., 2012) (Brodersen, 2005). Furthermore, SA is involved in regulation of flowering time, and in response to both biotic and abiotic stresses, high SA endogenous levels are observed in plants that transition earlier from bolting to flowering to increase chances of population survival (Martinez et al., 2003) (Lyons et al., 2015). This furthers the notion that SA promotes plant resource reallocation, diverting resources from increasing biomass to processes involved in reproduction, thus increasing the overall survival of the population rather than the individual. The outlier bolt height trend of intermediate *sid2* can be explained by the large standard deviation of 2.575±2.712cm. The sole significant differences between control *Col* and four other treatment groups, ASA concentration combinations may suggest that exposure or non-exposure to ASA has a greater impact on *Col* bolt height growth than different degrees of ASA exposure. Furthermore, all four ASA concentrations of *Col* experienced a net increase in mean bolt height and *sid2* exhibited various trends, suggesting a possible influence of genotype upon bolt height. However, standard deviation for the low, intermediate, and high *Col* were very large, and shared large amounts of overlap with the means and standard deviations of the *sid2*. This observation, and the lack of significant differences among low, intermediate, and high treatments further supports the possibility that changes in bolt height are most greatly influenced by the exposure or lack of exposure to ASA. Interestingly, trends for *M. persicae* populations and bolt growth for low and high *Col* plants appeared to be quite similar, both demonstrating gradual decreases in both response variables. However, the aforementioned relationship is only speculated based off of the minimal data in this study and should be further investigated with more specific future experiments.

BOLT HEIGHT ON APHID POPULATION

The intercept of the linear regression plot investigating possible effects of bolt height upon aphid population indicated that at changes in mean bolt height of 0cm, there was an expected significant mean aphid population increase of 2.07. Interpreting change in bolt height as a proxy for plant quality, this may suggest that *A. thaliana* in poor health experience an increase in aphid population. However, the absence of a significant slope suggests that there does not exist a relationship between the two variables, and it must be acknowledged that bolt height is an unreliable indicator of plant quality that can be affected by factors such as ascension line and and significant variation in the development rate of *A. thaliana* (Gnan, Marsh, and Kover, 2017) (Figure 7). Nevertheless, the significant intercept warrants further investigation, perhaps through consideration of the effects of interaction between genotype and height on aphid population.

CONCLUSION

Overall, interactions were found between both ASA concentration, genotype and time. These results led to the following conclusions: the null hypothesis that ASA concentration and genotype do not affect aphid population was rejected. Additionally, as a significant relationship was found between ASA concentration and bolt height, the null hypothesis that ASA concentration does not affect bolt height was rejected. These findings present further evidence for the impact of anthropogenic contaminants on not only the quality of A. thaliana and its associated immune response, but on the population growth of insects such as M. persicae. The irresponsible disposal of pharmaceuticals is having a significant impact on the environment. Thus, efforts should be made to implement new policies and procedures to limit pharmaceuticals such as ASA from entering the soil and water, and decrease anthropogenic ecological impacts.

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AUTHOR CONTRIBUTIONS

Rathod: Research contributions included: researched the study system of *A. thaliana* and *M. persicae* and experimental methods on exogenous delivery of substances to plants, and experimental design. Conducted research on SA and JA defense pathways. Authorship contributions included: wrote materials and methods section, and co-authored study system and results. Contributed to general editing.

Luu: Research contributions included: researched the SA and JA pathways, including mechanisms, genes induced, SAR, and local responses. Also researched crosstalk between SA and JA, and the impact on plant health and aphids. Conducted statistical analyses (ANOVA, ANCOVA, post hoc) for all data, created graphs alongside another group member to display trends of data. Authorship contributions included: contributed to results and discussion sections of the manuscript. Contributed to general edits and formatting for final manuscript.

Al Hashemi: Research contributions included: researched proper care and conditions for *A. thaliana*. Authorship contributions included: wrote the study system portion of the report, general editing and contributed to the discussion and conclusion.

Agueci: Research contributions included: Researched background information on *M. persicae* and *A. thaliana* (general traits, aphid and plant relationships with jasmonic/salicylic acid pathways, plant defence mechanisms against aphid feeding, the effects of acetylsalicylic acid on plants and aphids), experimental design. Authorship contributions included: formulation of appropriate research questions and hypotheses, created Excel graphs and analyzed their trends for the Results and Discussion sections. Contributed to edits and formulation of Abstract and Conclusion.

Dittrich: Research contributions included: investigated various anthropogenic ecological impacts to shape the research topic, followed by researching pharmaceutical and ASA impacts on water, soil, and plants once the topic was narrowed down, researched JA and SA pathways to determine which strains were appropriate for the experiment, and background research for the Introduction section of the manuscript. Authorship contributions included: compiled most of the abstract, wrote the Introduction section and part of the Materials/Methods section, wrote the references, and edited the rest of the manuscript.

REFERENCES

Association of Metropolitan Water Agencies. (2008). *Pharmaceuticals in the Water Environment*. National Association of Clean Water Agencies.

Brodersen, P., 2005. The role of salicylic acid in the induction of cell death in Arabidopsis acd11. *Plant Physiology*, 138, pp.1037-1045.

Cleuvers, M., 2004. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. *Ecotoxicology & Environmental Safety*, 59, pp.309-315.

Clissold, S.P., 1996. Aspirin and related derivatives of salicylic acid. *Drugs*, 4, pp.8-26.

Conrath, U., 2006. Systemic Acquired Resistance. *Plant Signal & Behaviour*, 1, pp.179-184.

Ghazijahani, N., Hadavi, E., & Jeong, B. R., 2014. Foliar sprays of citric acid and salicylic acid alter the pattern of root acquisition of some minerals in sweet basil (*Ocimum basilicum* L.). *Front Plant Sci,* p.5.

Gnan, S., Marsh, T., & Kover, P.X., 2017. Inflorescence photosynthetic contribution to fitness releases Arabidopsis thaliana plants from trade-off constraints on early flowering. *PLOS One*, 12, e0185835.

Haahr, M., 1998. *List randomiser*. Randomness and Integrity Services, Dublin.

Health Canada. (2012). *First Nations Food, Nutrition & Environment Study*. University of Ottawa, Ottawa.

Hullé, M. *et al.*, 2005. Geographical location, climate and land use influences on the phenology and numbers of the aphid, *Myzus persicae*, in Europe. *Journal of Biogeography*, 32, pp.615-632.

Kloas, W. *et al.*, 2009. Endocrine disruption in aquatic vertebrates. *Annals of the New York Academy of Sciences*, 1163, pp.187-200.

Kourneef et al., 2008. Towards a reporter system to identify regulators of cross-talk between salicylate and jasmonate signaling pathways in Arabidopsis. *Plant Signaling & Behaviour*, 3, pp.543-546.

Lajeunesse, A., Smyth, S., Barclay, K., Sauvé, S. and Gagnon, C., 2012. Distribution of antidepressant residues in wastewater and biosolids following different treatment processes by municipal wastewater treatment plants in Canada. Water Research, 46(17), pp.5600-5612.

Leon-Reyes et al., 2010. Salicylate-mediated suppression of jasmonate-responsive gene expression in Arabidopsis is targeted downstream of the jasmonate biosynthesis pathway. *Planta*, 232, pp.1423-1432.

Louis, J. and Shah J., 2013. *Arabidopsis thaliana - Myzus persicae* interaction: shaping the understanding of plant defence against phloem-feeding aphids. *Front Plant Sci*, 4, p.213.

Lyons, R. et al., 2015. Investigating the association between flowering time and defence in the *Arabidopsis thaliana-Fusarium oxysporum* interaction. *PLoS One*, p.10.

Martínez, C. et al., 2003. Salicylic acid regulates flowering time and links defence responses and reproductive development. *The Plant Journal*, 37, pp.209-217.

Metcalfe, C., Miao, X., Koenig, B. and Struger, J., 2003. Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the Lower Great Lakes, Canada. *Environmental Toxicology & Chemistry*, 22(12), p.2881.

Mewis, I., Appel, H., Hom, A., Raina, R. & Schultz, J., 2005. Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. *Plant Physiology*, 138, pp.1149-1162.

Moran, P and Thompson G.A., 2001. Molecular responses to aphid feeding in Arabidopsis in relation to plant defence pathways. *Plant Physiology*, 125, pp.1074-1085.

Morkunas, I., Mai, V. & Gabrys, B., 2011. Phytohormonal signaling in plant responses to aphid feeding. *Acta Physiologiae Plantarum*, 33, pp.2057-2073.

Nishimura, M.T. & Dangl, J.L., 2011. Arabidopsis and the plant immune system. *The Plant Journal*, 61, pp.1053-1066.

Orlando, E. and Ellestad, L., 2014. Sources, concentrations, and exposure effects of environmental gestagens on fish and other aquatic wildlife, with an emphasis on reproduction. *General and Comparative Endocrinology*, 203, pp.241-249.

Park, B. and Kidd, K., 2005. Effects of the synthetic estrogen ethinylestradiol on early life stages of mink frogs and green frogs in the wild and in situ. *Environmental Toxicology and Chemistry*, 24(8), p.2027.

R Development Core Team, 2016. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna.

Runnalls, T., Beresford, N., Losty, E., Scott, A. and Sumpter, J., 2013. Several synthetic progestins with different potencies adversely affect reproduction of fish. *Environmental Science & Technology*, 47(4), pp.2077-2084.

Schultz, M.M. et al., 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: Occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environmental Science & Technology*, 44, pp.1918-1925.

Serino, G. and Gusmaroli G., 2011. *Arabidopsis thaliana* as an Experimental Organism. *eLS*.

Senaratna et al., 2000. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regulation,* 30, pp.157-161.

Vogelmann et al., 2012. Early senescence and cell death in Arabidopsis saul1 mutants involves the PAD4-dependent salicylic acid pathway. *American Society of Plant Biologists*, 159, pp.1477-1487.

War, A.R. et al., 2012. Mechanisms of plant defence against insect herbivores. *Plant Signal & Behaviour,* 7, pp.1306-1320.

Wiermer, M., Feys, B. & Parker, J., 2005. Plant immunity: the EDS1 regulatory node. *Current Opinion in Plant Biology*, 8, p.383-389.