Tannins as a Pesticide: The Impact of Tannic Acid on the Growth Rates of *Myzus persicae* and *Arabidopsis thaliana*

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SUMMARY

We completed a study on the effectiveness of natural pesticides in lieu of artificial pesticides with the purpose of finding a more environmentally friendly alternative to pesticides. We investigated the interactions between the plant, *Arabidopsis thaliana*, and the herbivore, *Myzus persicae*, as well as the effect of tannic acid on these interactions. Our goal was to answer whether a dose of tannic acid would positively or negatively affect both the plant and the animal. This paper aims to determine if there are alternatives to pesticides which may be safer for the environment. We determined that, if the pesticide generated a positive or neutral effect on the plant and a negative effect on the herbivore, it could be considered an effective insecticide. After completion and analysis, it was determined that tannic acid is not an effective insecticide for our given study system at 0%, 1% and 3% concentrations. It had no effect on aphid population growth, and at a 3% concentration in the presence of aphids, a negative effect on plant growth, which presents itself as a possible future herbicide.

Received: 11/08/2018 **Accepted:** 03/12/2019 **Published:** 11/17/2019

Keywords: pesticide, insecticide, tannic acid, *Arabidopsis thaliana*, *Myzus persicae*, plant defence

INTRODUCTION

Pesticides are chemical compounds that humans have used as a convenient method to kill undesired plants, as a herbicide; or insects, as an insecticide (Aktar, Sengupta, and Chowdhury, 2009). Over two million tonnes of pesticides are used annually worldwide in the 3.342 trillion dollar agricultural industry, the majority of which are used to target insects (Worldbank, 2017). This subcategory of pesticides is called insecticides and indirectly cause an increase in the yield and quality of crops (Aktar, Sengupta, and Chowdhury, 2009). Despite these short-term benefits, the long-term effects of these artificial chemical pesticides are greatly concerning. For instance, many pesticides contain toxins that are dangerous to both humans and organisms beyond their intended target species (Nicolopoulou-Stamati, et al., 2016; Aktar, Sengupta, and Chowdhury, 2009).

Insecticides can also damage many non-target organisms in the environment (Ansari, Moraiet, and Ahmad, 2014). For example, insecticides can mix with main water systems through surface runoff, leading to contamination of aquatic ecosystems and drinking water (Mahmood et al., 2015; Aktar, Sengupta, and Chowdhury, 2009). This can negatively impact both aquatic organisms and humans. The chemicals in pesticides can also leach into soil and negatively affect many beneficial microorganisms that contribute to soil fertility, such as nitrogen-fixing bacteria. Furthermore, volatiles from the large-scale spraying of pesticides can also lead to air contamination (Aktar, Sengupta, and Chowdhury, 2009). There is also evidence that the wide use of insecticides has led to insecticide resistance in many insects (Ansari, Moraiet, and Ahmad, 2014).

Another alarming fact is that pesticides can have an impact on humans, negatively affecting health immediately. In fact, around 26 million people suffer from pesticide poisoning annually (Ansari, Moraiet, and Ahmad, 2014). Many workers in insecticide manufacturing report experiencing symptoms such as nausea, vomiting, and fatigue regularly (Mahmood et al., 2015). With long-term or frequent exposure, the chemical compounds can act as endocrine disruptors, leading to immune system suppression, reproductive abnormalities, and cancer (Mahmood et al., 2015; Nicolopoulou-Stamati et al., 2014). As of 2013, Canada is still using more synthetic pesticides than naturally-derived ones (Figure 1) (Government of Canada, 2013). For this reason, it is crucial to find alternative pesticides for commercial use in Canada that are safer for the environment and human health.

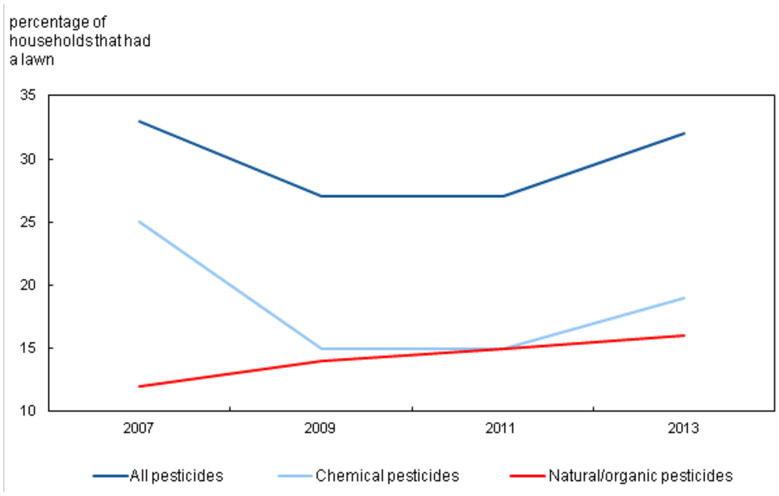

Figure 1: A diagram comparing the percentage of chemical and natural pesticide usage in Canada from 2007 to 2013 (Government of Canada, 2013).

Tannic Acid

We propose the use of tannic acid ($C_{76}H_{52}O_{46}$) as a naturally derived alternative to insecticides. It has the potential to be a good alternative to artificial insecticides since tannic acid is already naturally produced by many woody plants therefore not requiring chemical manufacturing. However, one must also consider that the cost and effort to extract tannic acids from plants may be great (Karchesy, Kelsey, and González-Hernández 2018). They are commonly found in several different types of plant life, including *Castanea sativa* (sweet chestnut), *Quercus infectoria* (Aleppo oak), and *Rhus spp.* (sumach) along with several other organisms (European Safety Authority, 2014). The tannins can be extracted easily using solvents such as 80% acetone or an alcohol ether mixture (Karamać et al., 2007; Gino, 1951). There are several studies that demonstrate the benefits of using tannic acid. For instance, humans are already accustomed to metabolizing tannin-rich foods, so it will have a minimal impact on human health, especially at low concentrations (Chung et al., 1998). There is also evidence that tannins are a more environmentally friendly alternative to bactericides when treating leather (Zengin et al., 2014), and it has already been proposed to be an alternative fungicide (Forrer et al., 2014).

Tannins are naturally produced by plants as a defence mechanism against plant-feeding insects (War et al., 2012). The chemical defends against insects through a variety of mechanisms. Firstly, tannins oxidize within the guts of insects due to its highly acidic climate, then bind to various essential amino acids, such as important digestive enzymes, within the insect's gut (Barbehenn & Constabel, 2011). Tannins can cause significant nutrient loss by binding to many lipids and carbohydrates that insects ingest, leading to decreased digestibility of these molecules (Barbehenn and Constabel, 2011). In addition, there is evidence that tannins inhibit insect development by causing the formation of midgut lesions (War et al., 2012), likely due to the semiquinone and quinone free radicals released when tannins oxidize (Barbehenn and Constabel, 2011). Furthermore, tannins also act as feeding deterrents to insects due to their bitter taste (War et al., 2012).

Tannic acid is already used as a fungicide, inhibiting bacterial growth (Forrer et al., 2014). Therefore, we set out to investigate tannic acid's potential as an insecticide against herbivores, such as the green peach aphid (*Myzus persicae*), a commonly found pest. While tannic acid has been shown to reduce fitness in grasshoppers, little has been done to demonstrate its effect on aphids, and especially how it jointly interacts with both plant and pest. Unlike many other pesticides, it does not present a considerable risk to human health, such as having no mutagenicity *in vivo*, or carcinogenic sources (Onodera et al., 1994). Due to its natural presence in the environment, there is not a major concern for it to be harmful for the environment (European Safety Authority, 2014).

The purpose of our study was to investigate the relationship between a pesticide (tannic acid) and the health of both an animal (*Myzus persicae*) and the plant (*Arabidopsis thaliana*). We hope to answer the question: what is the effect of different concentrations of tannic acid on *M. persicae* population growth rate and *A. thaliana* plant growth rate? Our hypothesis is that, by treating *A. thaliana* with varying tannic acid concentrations, the overall health of the plant will not be affected. As well, the tannic acid will affect *M. persicae* negatively, decreasing population growth rate over time. Conversely, our null hypothesis is that neither the growth rates of *A. thaliana* nor *M. persicae* will be affected by tannic acid concentrations. This experiment was conducted to investigate whether tannic acid could act as a natural and environmentally friendly pesticide. More specifically, as tannic acid is used by some plants as a defence mechanism, we wanted to demonstrate whether applying this compound to the surface of *A. thaliana* could aid in its defence against *M. persicae*.

MATERIALS & METHODS

STUDY SPECIES: MYZUS PERSICAE

This section aims to provide background information on the species *M. persicae*, commonly referred to as the green peach aphid. It is a model organism for research regarding plant and animal interactions. *M. persicae* are known to feed on over 40 different families of plants, including *A. thaliana* (CAB International, 2018). Illumina sequencing technology has been used to compare the genomic differences between adult and nymph *M. persicae*. The life stages of *M. persicae* vary in cuticle formation, detoxification, hormone production, and metabolism, which has resulted in 2244 genes being expressed differently between the two stages (Ji et al., 2016). This can affect the aphid's feeding and migratory patterns on the plant. *M. Persicae* has several different life stages; egg, nymph, and adult. The adult stage can be divided into two subgroups: viviparous and alate. The viviparous are unwinged and able to give birth while alate is the winged morph. A study by Srinivasan and Brisson discusses phenotypic plasticity, the process in which aphid females send signals that can affect the development of their offspring (2011). The life cycle of *M. persicae* must be taken into consideration during this study, as different stages of life may affect plant growth differently. The youngest life stage is nymphs, which are green or green-yellow in colour. They can grow into viviparous adults that are capable of asexual reproduction (Capinera, 2001). This is an effective model system for studying the effects of environmental cues (e.g. tannic acid concentrations) on animal growth patterns and phenotypic plasticity (Srinivasan and Brisson, 2011). We obtained our aphid population from other *A. thaliana* plants hosting aphids in a laboratory at McMaster University, under the direction of Dr. Chad Harvey.

STUDY SPECIES: ARABIDOPSIS THALIANA

The plant species chosen for this study is *A. thaliana*, commonly used in a variety of biological experiments. It is an ideal plant for our purposes, as there is extensive background already known about the plant's genome and structure (Van Norman and Benfey, 2010). The plant's size ranged from 1.1 cm to 5.7 cm tall, allowing for many plants to be stored in limited space. Furthermore, the life stages are easy to distinguish through observation. Our study examines plants in rosette, bolting, and flowering stages. Also, the life cycle of *A. thaliana* is short, at approximately six weeks, allowing for easy study of how various factors affect plant growth over time (Koornneef and Meinke, 2010). This species is very straightforward to maintain and can be found in many locations across North America, Europe, Asia, and Africa (Clarke, 1993).

INTERACTIONS BETWEEN A. THALIANA AND M. PERSICAE

In the case of *A. thaliana* and *M. persicae*, the herbivore requires the plant as a habitat and source of nutrition in the context of our study. The plant, however, does not require the herbivore and is constantly trying to ensure that the herbivore does not kill it, as herbivores regularly do to plant species. This is consumption, therefore, since the plant does not require or benefit from the presence of the herbivore. In fact, the plant is consistently trying to eliminate the aphids from its environment through its own defenses. *A. thaliana* has mechanical defenses, specifically trichomes, to protect itself from herbivores (Bruner, 2009). The trichomes are modified epidermal cells, specially designed to eliminate herbivores. This is evident when we observe where *M. persicae* resides on the plant and see that it abides mostly under the leaves and on the stem where trichomes are rare. We are also aware that if plants are invaded by herbivores, their jasmonic acid pathway will be suppressed by the active salicylic acid pathway (Capinera, 2001). This would decrease the defense against bacterial pathogens while increasing defense against herbivores.

TANNIC ACID TREATMENT

All plants used for the experiment were separated by applied insecticide amount: 0%, 1%, and 3% w/w tannic acid, whereby each group had equal number of aphid and no aphid treatments. These treatment concentrations were chosen to investigate the lowest concentration of tannic acid which would influence our study species. Following methods similar to Bien (2016), three solutions were dissolved using pure, powdered tannic acid in water. They were then administered to the plant by spraying each plant three times from a spray bottle approximately 5 cm away. This treatment was administered only once, at the beginning of the experiment.

EXPERIMENTAL DESIGN

Thirty-six *A. thaliana* plants were used in this experiment. To minimize potential block effects that could be caused by the separation of the plants into three covered trays, we randomized the placement of the different treatments. Individual trays received two plants of each treatment type: each with different acid concentrations with either no aphids or three aphids initially. A random number generator (Accessed through website www.random.org) was used to decide in which position each plant would be placed within the tray. Each possible position for the plant was assigned a number. Then, through the number generator, we randomly designated a position for each plant. Then, cardboard dividers were placed to separate the plants from one another, spanning from the bottom of the tray to around plant-height to avoid blockage of sunlight. This experiment design method was selected to minimize any potential block effects and ensured each treatment would have the same probability of being affected by confounding factors such as proximity to light source, proximity to plants with aphids, and potential temperature differences. The plants were labelled based on the aphid treatments (yellow tape to indicate presence of aphids or orange tape to indicate absence of aphids) and the tannic acid concentrations (blue tape to indicate 0%, green tape to indicate 1%, and red tape to indicate 3% tannic acid) (Figure 2).

Figure 2: There are six treatment groups within our experiment. The blue labels represent the control treatment (i.e. no tannic acid), the green labels indicate a 1% tannic acid solution was applied, and a red label indicated that a 3% solution of tannic acid was applied. The yellow labels represent the aphid treatment. These six treatment groups were placed in three different trays in a randomly arranged mixing of the categories seen above.

This may have contributed to observer bias; however, it is believed that since our observations were numerical in nature, this would not have affected our data to a great extent. The 1% tannic acid was made through serial dilutions of the 3% stock solution. We varied the concentration to both analyze the effects of different concentrations as well as to potentially propose an optimal concentration for the most effectiveness.

After the plants had been inoculated with three aphids on each plant with orange tape, they were placed in similar lighting conditions: indoors, beside natural light, and covered in a transparent lid to prevent cross-contamination of aphids. Unfortunately, cross-contamination could not be prevented within trays. Over the course of 12 days (September 20th, 2018 to October 2nd, 2018), maximum plant height, maximum plant diameter, and aphid count, along with qualitative observations of leaf discolouration and bolting on each plant were measured and recorded only 9 of those days due to limited access to the laboratory room. More specifically, observations were taken on the day they were inoculated (day 0), then day 1, 4, 5, 6, 7, 8, 11, and 12. After 7 days of the experiment, each plant was watered with 6 mL of water directly into the soil so as to not interfere with the tannic acid treatment on the stem and leaves.

Aphid population growth rate was determined by calculating the slope of the aphid count over time for each individual plant. This was necessary to analyze due to the uneven time separation of each aphid count measure. This method of calculating aphid population growth rate can account for that potential confounding factor and minimize measurement imprecision that could occur day to day. These imprecisions were caused by different observers and the possibility for counting error.

Plant growth rate was determined first by calculating plant conical volume, using both plant height and diameter in methods similar to those proposed by Pontailler et al. (1997) (Box 1). Both plant height and plant diameter were determined to be crucial to consider because there were some healthy plants that did not bolt but experienced width growth, and there were also healthy plants that bolted and did not grow in diameter. A slope was then determined from plant conical volume change over time for each individual plant.

$$\text{Plant Conical Volume} = 1/3\pi \left(\frac{\text{Plant Diameter}}{2}\right)^2 (\text{Plant Height})$$

Box 1: The equation for plant conical volume is used to combine the variables of plant diameter and plant diameter (Pontailler et al., 1997).

STATISTICAL METHODS USING R

Before statistically modelling the effect of tannic acid on plant growth rate and aphid population growth rate, another variable that must be considered is the potential interacting effect of plant growth rate and aphid population growth rate. This was investigated using a linear regression between aphid population growth rate and plant growth rate of all the treatments combined.

A two-way analysis of variance was used to determine the possibility of tannic acid treatment levels and aphid treatment levels interacting to influence plant growth rate. Next, a Tukey HSD post-hoc test was performed to isolate the differences of means and investigate significance.

Next, the relationship between aphid population growth rate and tannic acid treatment levels was investigated using analysis of variance solely in the plant group which was inoculated with aphids. The effectiveness of the tannic acid treatment as a pesticide can be determined by analyzing the difference between the means of all three levels of acid treatment on the aphid population growth rate. A post-hoc test was not performed since there was no significant correlation.

RESULTS

OBSERVATIONS

Over the course of 11 days, some visual changes were observed in the plant and aphid population growth. Over time, 27 of the 36 plants developed yellow or shriveled leaves. Aphids were often found on the stem of the bolted plants or under the leaves for plants in both the bolted and rosette stages (i.e. where there was little or no tannic acid treatment if applicable). The greatest density of aphids was often found at the base of the stems. The aphids found were most often nymphs; however, several viviparous adults and several alates were found as well. As a result, these winged aphids would have been able to migrate and colonize other plants in the vicinity, including plants that originally had no aphids (Figure 3). This was observed when plants which initially were not inoculated with aphids were found to have aphids by the end of the experiment.

Figure 3: This *Arabidopsis thaliana* plant was treated with 3% tannic acid solution and populated by aphids. It has visible damage such as dark brown spots, yellowing, and loss of structure to the leaves that was common to plants of this treatment group.

Many of the plants exposed to the tannic acid treatment, both at 1% and 3% concentrations, had dark brown spots visible on the leaves. These were only visible on the leaves which were already present at the time of treatment (i.e. none of the new leaves were affected). These brown spots were visible after one day of treatment on several plants; however, they were more apparent later in the trials. Furthermore, the plants with the 0% tannic acid treatment did not have these dark brown spots for the duration of the experiment. Almost all of the plants with the 3% concentration treatment had brown spots on their leaves.

PLANT GROWTH

Plant growth rates were not significantly related to aphid population growth rate within the study ($R^2=0.01363$, F(1,16)=1.235, p>0.1). This was investigated solely on the aphid treatment level because even though the non-aphid treatment had some aphids due to contamination, there were too few to appropriately consider this relationship. This allows us to investigate plant growth along with aphid treatment levels independently of aphid population growth rate (Figure 4).

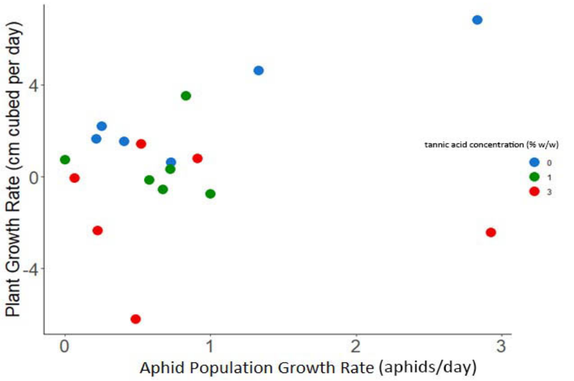

Figure 4: Within the aphid treatment and all three acid concentrations, plant growth rate and aphid population growth rate were plotted together and found to not have a significant correlation. Tannic acid concentration is categorized in percentage by weight (0%, 1%, and 3%).

Plant growth was significantly affected by the interacting effect of tannic acid and the aphid treatment (F(2,30)=5.794, p=0.00745). In the non-aphid treatment, there were no significant differences between the 0% (M=0.61 cm^3/day, SE=.32), 1% (M=2.37 cm^3/day, SE=0.64) and 3% (M=0.86 cm^3/day, SE=0.43) levels of tannic acid on plant growth (p>0.1). There was a significant difference between 3% and 0% for the aphid treatment (p<0.05). The 0% tannic acid aphid treatment (M=2.92 cm^3/day, SE=0.96) had a mean plant growth rate that was 4.38 cm^3/day higher than the 3% tannic acid aphid treatment (M=-1.46 cm^3/day, SE=1.15). Furthermore, the negative mean growth rate shows that, on average, the plant volume decreased over time under the presence of tannic acid. However, there were no significant results between 0% and 1% (M=0.72, SE=0.65) for the aphid treatment (p=0.2438275) as well as 1% and 3% for the aphid treatment (p=0.4287740) (Figure 5).

APHID POPULATION GROWTH

The growth rate of the aphid population was not found to be affected by the growth rate of the plant, so aphid population growth and its connection with tannic acid was examined independently of plant growth solely in the group

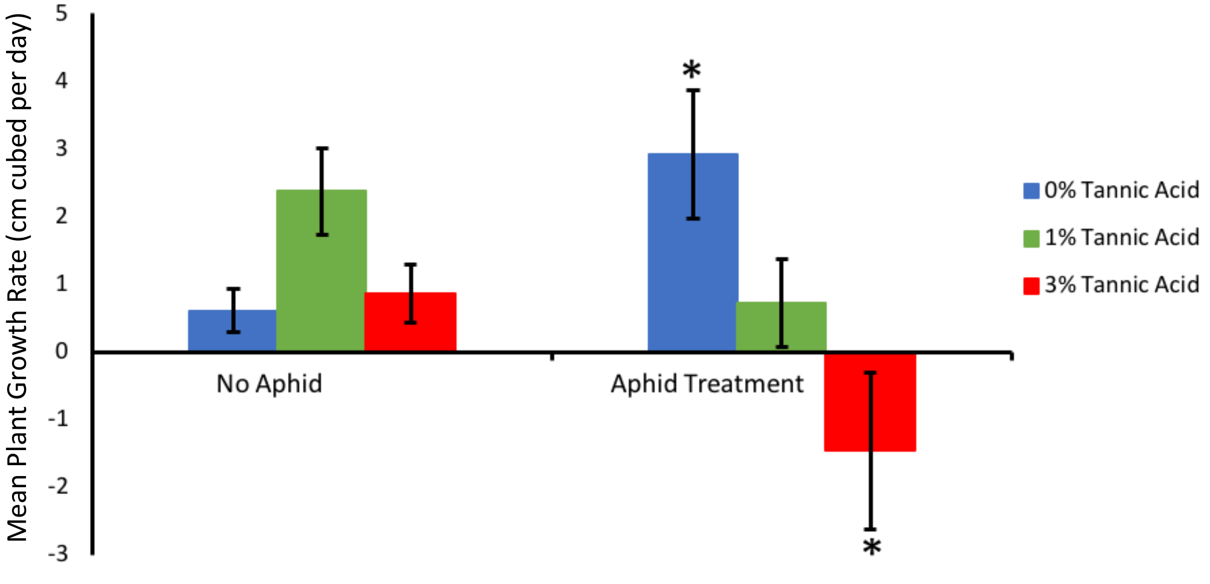

Figure 5: The effect of tannic acid concentration on mean plant growth rate. There is a significant difference of means between 0% and 3% for the aphid treatment. The mean plant growth rate of 3% was 4.38 cm^3/day less than the 0% concentration. A negative growth rate is seen solely for the 3% tannic acid within the aphid treatment.
* denotes significance of p <0.05.

which was inoculated with aphids. Aphid population growth rate was not significantly related to tannic acid concentration at any concentration (F(2,15)=0.2227, p=0.8029) between any of the concentration levels: 0% (M=0.61, SE=0.32), 1% (M=2.37, SE=0.64), and 3% (M=0.86, SE=0.43) (Figure 6).

CONFOUNDING FACTORS

Several unforeseen elements of our experiment may have affected our results. Moss was present on the soil of some of the samples. Alates were able to migrate between plants, and consequently, likely colonized other plants, as most of the plants that were not inoculated with aphids had aphids at the end of the experiment, and therefore our non-aphid treatment was determined to have been contaminated.

DISCUSSION

As indicated by the results of our experiment, tannic acid is not a suitable pesticide within our study system, though many plants also use tannic acid as a chemical defense (Clausen et al., 1992).

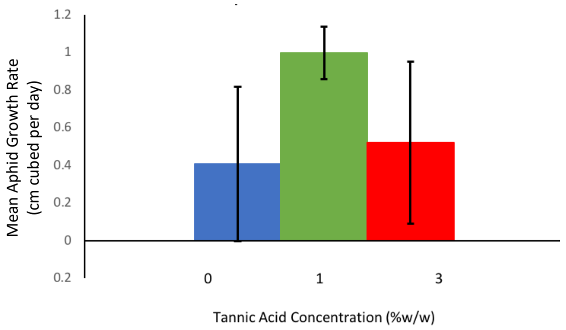

Figure 6: There is no effect of tannic acid on mean aphid population growth rate. All the aphid population growth rates are positive, demonstrating that aphids increase in population over time.

Tannins are naturally a difficult chemical to digest for herbivores, decreasing the nutritional value of the plant for the herbivore. This is one of the main reasons we wanted to test tannins on the plant. Tannins negatively affected the plant invesitaged in our study, even though they naturally occur in many other plants and are used to harm herbivorous predators. The aphids in our study were not negatively affected by tannins, contrary to what was expected. Perhaps they were able to overcome this issue because commercialized tannins have been known to have different dietary effects on herbivores (Hagerman et al., 1992). In fact, due to insecticide overuse, some species of insects have evolved gut adaptations, such as extreme pH tolerance and preventing tannins from interacting with digestion (Martin, Rockholm, and Martin, 1985). However, it must be noted that tannins are a digestibility reducer and have not been found to affect sucking insects, such as aphids, in the same way as they affect insects which chew on plants (Stienezen, M., et al., 1996). Finally, topical application such as in our experiment interacts differently with the aphids as would typical ingesting of tannins that come from within the plant itself.

Tannic acid did not increase plant growth but instead damaged the plants when coupled with an aphid attack. The aphids induced a stressful environment in which the plant is taxed for resources. This occurs when a tannic acid concentration of at least 3% is also added to the plant; consequently, it cannot handle the extra stress and its growth rate substantially decreases. These results support the *Plant Stress Hypothesis*, whereby stresses, such as insect herbivory, act as a resource sink so that the plant is not able to properly protect itself from the tannic acid and crop damage ensues (Joern and Mole, 2005).

FUTURE STUDIES

One of the main confounding factors in this study was contamination of aphids on the no aphid control group. In future studies, it would be beneficial to create an environment where the individual *A. thaliana* samples are completely isolated to better analyze the independent effects of aphids and tannic acid concentration on plant growth rate. This experiment also did not account for the effects of tannic acid wearing off over time. The tannic acid spray was not reapplied as it might have affected the aphid populations on the plants. It would be interesting, instead, to find a mechanism to maintain a constant level of tannic acid present on the plant without disturbing the aphids.

Since the tannic acid was only effective as a herbicide when in the presence of aphids at higher populations, it would be of interest to test stronger concentrations of the acid without aphids. This may provide a simpler solution to a herbicide with the intent of eliminating unwanted plants, such as invaders, or even weeds in a garden. The resulting herbicide would be a natural one, with fewer negative effects on the environment and humans than an artificial pesticide (Okuda, 2005).

CONCLUSIONS

From our study, we have been able to conclude that tannic acid is not an effective insecticide for the plant *A. thaliana* with respect to the herbivore *M. persicae*. Although the acid did appear to be effective under the condition that it was at a high concentration (3% in this study) and inhabited with pests, it still results in the conclusion that the tannic acid negatively affected the plant growth. This is not ideal for an insecticide as the plant would die while the pest would thrive. It may be possible that in another study system, this pesticide would prove effective. For example, the acacia plant utilizes tannins as a natural form of defense against herbivores (Elgailani and Ishak, 2014). It may be hypothesized that if tannins were to be sprayed onto an acacia plant, the plant may have an increased defense against herbivores. If this were to occur, the tannins would be considered an effective natural insecticide. This would encourage further studies into tannins and other study systems.

These results, however, should not discourage further research for other natural pesticides, even those which are used in plant defenses. One could look into another plant defense mechanism which would enhance the plant's defense against aphids and not harm the individual plant. Studies could also be designed to test if higher concentrations of tannic acid could damage the plant enough to kill it without the presence of aphids.

We have determined that tannic acid could be a potential effective herbicide instead of insecticide at the tested concentrations. This could lead to new ideas in the field of invasive species management, as it is naturally produced and not harmful to plant or human health. For example, if we aimed to be rid of an invasive species, we could send a herbivore (such as *M. persicae*) onto the plant and then spray the plant with tannic acid. From our results, the tannic acid must be in the presence of aphids to damage the plant enough that it dies. These conclusions allow room to create new studies into the efficacy of tannic acid or if there is another natural compound which would more effectively impede plant growth rate.

Based on our finding that 3% tannic acid damages plants and decreases plant growth rate when in the presence of aphids, we suggest that tannic acid has the potential to be used as a herbicide instead of an insecticide. If a population of plants is infested by aphids, applying tannic acid to the leaves would decrease the plant growth rate if the desired solution to the infestation is to kill the plants.

ACKNOWLEDGMENTS

We would like to acknowledge Dr. Chad Harvey for his teachings about plant-animal interactions as well as his guidance in the experiment, Dr. George Dragomir for his clarification of growth rates, and Noah Houpt for his helpful advice and support throughout the process.

Author Contributions

All authors contributed to design of study, research, statistical analysis, methods, materials, and manuscript writing.

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