Host plant choice in spider mites – does mother know best?

Thomas Cupido, s3484157
Konstantinos Kyriakou, s4703634
Wessel Warnders, s4732332

Supervisor: Martijn Egas

ABSTRACT

When learning provides an individual with an increase in reproductive success it can be described as adaptive learning. This study investigates adaptive learning in two-spotted spider mites (*Tetranychus urticae*) through a series of experiments, aimed at understanding to which extent preference and aversion influences host plant choice between lemon (*Citrus limon*), potato (*Solanum tuberosum*), and rose (*Rosa spp.*). The first experiment looked at the fitness associated with different host plant species. The second experiment looked at the induction of preference when presented with two substrates. The third experiment was an assessment of the underlying mechanisms behind the choice of the mites (true preference or aversion towards specific substrates), when presented with a familiar and a new plant.

The results indicated that lemon was significantly detrimental towards the fitness of the spider, whereas potato and rose did not have significant effects on their reproductive success. The spider mites displayed a significant disliking of lemon as a feeding and oviposition substrate, proving that mites are capable of adaptive learning when combined with the results for fitness.

The final experiment showed that the differences between preference and aversion when it comes to deciding on a host plant are insignificant, hinting towards a more complex and intricate relationship between these two underlying mechanisms.

Though many of the results obtained were not statistically significant, this report aims to offer insights for future research investigating the ecological and evolutionary importance of adaptive learning, as well as the mechanisms behind it.

INTRODUCTION

Despite being an extensively researched topic, learning is a concept that lacks a comprehensive definition (Barron et al., 2015). Learning is complex and multi-faceted (Nieberding et al., 2018), with previous research suggesting that its various aspects have evolved under selection (Papaj & Prokopy, 1989). One of the characteristics of learning is the change in behavior of an individual in a consistent manner as a result of experience (Papaj & Prokopy, 1989). Combining this with its capability to facilitate adaptation, adaptive learning is defined as a change in behavior resulting from previous experiences which lead to an increase in fitness (Egas & Sabelis, 2001).

Plant defenses have varying effects on the fitness of animals that feed on them (Santamaría et al., 2020). Adaptations such as chemical and physical defenses, and nutritional quality, are the result of an ongoing evolutionary arms race between plants and herbivores (Marinosci et al., 2015). These plant adaptations affect the fitness of herbivorous arthropods that feed on them by affecting several of their life-history traits, like fecundity (Awmack & Leather, 2002), larval development time (Ojala et al., 2005), and adult longevity (Hong et al., 2019). Therefore, learning to select for less hostile host plants could provide polyphagous herbivorous arthropods with an increase in their reproductive success.

Two-spotted spider mites (*Tetranychus urticae*) are polyphagous herbivorous chelicerates which have displayed adaptive learning in prior research. Specifically, *T. urticae* have been shown to be able to develop the ability to discriminate between host plants based on species (Egas & Sabelis, 2001), plant quality (Egas et al., 2003), as well as transgenicity (Rovenská et al., 2005), with said preferences being associated with a higher fitness. This, in combination with their physical features such as their small size and short life cycle (Capinera, 2008), makes *T. urticae* an excellent specimen for the study of adaptive learning in the context of development of food preference.

The objective of our research is to investigate adaptive learning in two-spotted spider mites through a series of experiments, using three host plant candidates (*Citrus limon*, *Solanum tuberosum*, and *Rosa spp.*). Firstly, an experiment shall be conducted comparing the implications that each host plant candidate has on the reproductive performance of the two-spotted spider mite. Oviposition and hatch rate will be studied and used as proxies for reproductive success. Secondly, the induction of preference for a host plant shall be examined to confirm the findings of previous studies suggesting that this can be a learned behavior. Lastly, in the case where induction of preference is observed, we aim to discover whether this choice is the result of preference towards one host plant, an aversion towards another, or both. The research question we aim to answer through this study is:

To what extent is adaptive learning a consequence of preference or aversion towards a specific host plant in two-spotted spider mites?

MATERIALS AND METHODS

Two-spotted spider mites

Adult *T. urticae* are 0.4-0.5 mm long, with females being larger in size (Capinera, 2008). The species possesses a wide range of host plants it feeds on, with more than 1,000 hosts belonging to more than 250 families (Van Leeuwen et al., 2015). They colonize leaves and feed on their mesophyll layer through the utilization of a retractable stylet (Santamaría et al., 2020). They subsequently cover the leaf in a silk web which provides them with refuge and a substrate for oviposition (Clotuche et al., 2011). Spider mites reproduce through arrhenotoky (males are haploid, females are diploid) and have extremely female-biased sex ratios, with female:male ratios ranging from 2:1 to 9:1 (Macke et al., 2010). Female spider mites lay approximately 60-120 eggs during their lifetime and oviposit at a rate of roughly 5-6 eggs per day (Capinera, 2008). The mites used originate from a stock population whose lineage had been exclusively reared on bean (*Phasoleus vulgaris*) leaves.

Selection of plants

The selection of plant species for experimentation was guided by the three categories characterizing arthropod-plant interactions: antixenosis, antibiosis, and tolerance (Santamaria et al., 2020). Antixenosis represents a feeding aversion characterized by deterring factors, antibiosis is the presence of chemical and morphological factors which negatively impact the biology of the spider mites, and tolerance is the plant’s ability to recover from any damage induced by the pest (Santamaría et al., 2020). A plant was selected to represent each category of these plant defenses.

The lemon tree (*C. limon*) exhibits antixenosis in the form of herbivore-induced plant volatiles which have a repellent effect on *T. urticae* (Agut et al., 2015). The potato plant (*S. tuberosum*) displays antibiosis, as it is a solanaceous plant, possessing leaves containing steroidal alkaloids (Friedman, 2006), which can be detrimental to herbivorous arthropods (Altesor et al., 2014). Rose (*Rosa spp.*) was selected as the plant species representing tolerance, due to its observed adaptive responses to herbivory (Meena et al., 2013). In the context of the study investigating reproductive performance *P. vulgaris* serves as a control, as it can be assumed that it provides the spider mites used with the highest fitness, due to evolutionary host adaptation (Rioja et al., 2017). The plants were kept in the lab for the duration of the experiment, and received water regularly.

Preparation of spider mites

Female mites were collected from the aforementioned stock population, which consisted of both male and female individuals, to ensure that all females extracted were fertilized, and thus capable of bearing female offspring.

The target for the experiment investigating the induction of preference was to acquire 150 female, developmentally-synchronized spider mites. In order to achieve this ~225 eggs would be required, as the lowest expected female:male ratio is 2:1 (Macke., 2010), and therefore 60 females were used as each spider mite would lay ~5 eggs per day (Capinera, 2008). The spider mites were then placed on bean leaf disks, 1.6 mm in diameter, on wet cotton wool. The use of leaf disks instead of whole leaves increases the likelihood of the spider mites surviving until the end of the experiment as research by Kavousi et al. (2009) found that spider mites reared on leaf disks for the entirety of their life had a longer lifespan and a slower growth rate. Additionally, as bean leaves are not flat, using leaf disks minimizes the curvature of the surface accessible to the spider mites, decreasing the likelihood of them crawling on the underside of the leaf where their trichomes can potentially tangle with the cotton wool.

A similar procedure was followed for the preparation of the spider mites for the experiment investigating reproductive success, with the only difference being the species of plant the leaf disks originate from. This experiment looks at how different substrates (*C. limon*, *S. tuberosum*, *Rosa spp. P. vulgaris*) affect oviposition and hatch rate. Therefore six 1.6 mm leaf disks were prepared for each substrate. 108 adult females were selected from the stock population, with 27 being used for each substrate. Each leaf disk received four or five mites.

A protocol devised by Suzuki et al. (2017) was used as a basis for the formulation of our own protocol for the handling and preparation of the spider mites, designed to better match the equipment and materials available to us, as well as the requirements of our experiment. Stepwise procedure:

1. A Petri dish is filled with a flat layer of moist cotton wool.
2. Create holes on the lid of the Petri dish to avoid condensation forming, using a soldering iron.
3. Prepare leaf disks 16mm in diameter using a hollow hole punch.
4. Place the leaf disks on the cotton wool and gently press down to make them as flat as possible.
5. Transfer adult female spider mites from the stock population to the leaf disks using a fine paintbrush to pick them up.
6. Incubate the spider mites for 48 hours at 23°C, with 16 hours of light exposure.
7. Using a fine paintbrush remove the adult female mites from the leaf disks, keeping only the eggs they have laid.
8. Place the Petri dishes back into the incubator without changing the conditions of incubation.
9. Monitor larval emergence and development on a 24 hour basis to minimize likelihood of systematic errors.
10. In the case that the leaf disks desiccate, add additional leaf disks to provide any hatched larvae with an adequate source of nutrition.

Test for reproductive performance

This test aims to quantify the effects each substrate has on the reproductive performance of the spider mites. To do so, oviposition and hatch rate are used as proxies for fitness. The female spider mites were given 48 hours to oviposit on the substrate they were placed on, after which oviposition was measured, and the female spider mites were removed. Subsequently, the eggs were left to incubate over a span of 10 days, with larval emergence being monitored in 24 hour intervals.

Investigating induction of preference

Despite learning in the context of host plant choice having been observed before in *T. urticae*, this experiment aims to repeat and confirm the findings of previous studies such as Egas & Sabelis (2001). To do so, choice arenas were formed. These are leaf disks, 10 mm in diameter, consisting of two half disks, each originating from a different plant species, with the two halves being connected by a pin with a glass head. Each choice arena held one spider miter, which it forced into making a choice on which side of the arena it would rather feed on and oviposit.

Three different choice arenas were prepared: potato - rose; potato - lemon; rose - lemon. To keep the pins in place and the leaf disks from desiccating, they were once again placed on a layer of flat, moist cotton wool. A female mite was transferred to the head of each pin, and given 24 hours to crawl down onto the leaf disks, scout both sides of the arena, and decide which one it preferred. Preference was determined by looking at which half disk experienced the most oviposition. In the case where there was no, or equal oviposition, preference was determined by the position of the mite on the leaf disk after the 24 hours had expired. After establishing themselves on either side of the disks, the spider mites proceed with web production (Gutierrez, 1985), thus making them stay in relatively the same position.

After the expiration of 24 hours, the mites were moved from their leaf disk to the head of a pin connected to a new choice arena of the same treatment. This was repeated for 3 days, with the spider mites incubating under the aforementioned conditions (23°C, 16 hours of light exposure) during these intervals. Spider mites which died during these intervals or during handling, were excluded from further analysis.

Preference vs. Aversion

After the completion of the first three days, all surviving mites proceeded to the final experiment which investigates to what extent preference and aversion learning influence the induction of choice. Each treatment from the previous experiment had its mites placed on new choice arenas, where one of the two halves consisted of a substrate they were familiar with, while the other half consisted of a substrate they had not encountered before (e.g. half the mites from the lemon | rose treatment were presented with lemon | potato choice arenas, while the other half were presented with potato | rose choice arenas). For this experiment, the spider mites were given ~12 hours to decide which substrate they prefer, and preference was measured using the same technique as the earlier experiment.

RESULTS

Preference experiment preparations

Of the desired 150 female developmentally-synchronized mites, only 12 hatched and reached adulthood by the time when experimentation was meant to take place. This was mostly due to a slower than expected, and heterogeneous rate of development, in combination with very high numbers of mortalities and eggs that did not hatch. This could be attributed to a malfunction of the incubator in which the spider mites were kept, as the settings had been reset to default (20°C, no light exposure). This malfunction was noticed four days after when it had occurred, and the desired settings (23°C, 16 hours of light exposure) were restored.

Due to this, an urgent extraction of 60 adult female spider mites from the stock population took place in order to obtain a large sample size to ensure sufficient replications per treatment. This need for additional spider mites led to a compromise being made in terms of developmental synchronization, as the age of the newly extracted spider mites was unknown. As a result, 72 mites were used in the subsequent experiment (n = 24, per treatment).

Reproductive performance

Bean leaves yielded the highest results for both fitness measures, with n = 42 eggs oviposited, and a hatch rate of 97.6% after the 10 days it was monitored. Out of the three host plants being investigated further, lemon leaves proved to be the most hostile towards the spider mites, with the lowest total oviposition (n = 14), as well as the lowest hatch rate after 10 days (7.1% of eggs hatched). Mites on potato (n = 28) performed better than mites on rose (n = 26) in terms of oviposition, whereas the opposite was true when looking at hatch rate (60.7% for potato, 76.9% for rose).

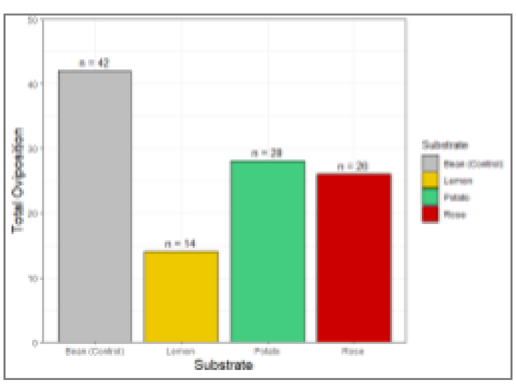

Figure 1 *Total oviposition per substrate.*

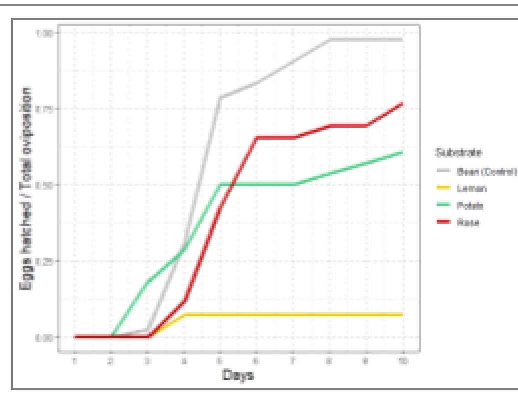

Figure 2 *Hatch rate for different substrates.*

The obtained values for hatch rates ranged between 0 and 1, and therefore had to undergo an arcsine transformation, in order for their significance to be assessed using repeated measures ANOVA. The statistical test proved that differences in hatch rate between the substrates were significant (P = 8.38e-09)

To determine significant differences between specific substrates, Tukey's HSD (Honestly Significant Difference) test, with the “Bonferroni” adjustment method was utilized (**Table 1**). The results of the test indicated that the differences in hatch rate between bean, potato, and rose, were not significant, while lemon had a significant difference in hatch rate when compared to all other substrates.

Substrate 1 - Substrate 2	**P-value**
Bean - Lemon	0.00405
Bean - Potato	0.471
Lemon - Potato	0.0248
Bean - Rose	0.395
Lemon - Rose	0.0336
Potato - Rose	0.895

Table 1. *Results of Tukey’s HSD test.* All data used underwent an arcsine transformation.

Induction of preference

In order to assess the induction of preference in spider mites, choice arenas were created, presenting each individual with two out of the three substrates being examined, leading to the formation of the following three treatments: lemon | potato, lemon | rose, potato | rose. As the results of preference per treatment were binary (preference for one substrate, or the other), binomial tests were carried out to determine whether a significant preference was present in each treatment. Significant results were obtained for the lemon | potato (P = 3.3532e-07) and lemon | rose (P = 5.1002e-09) treatments, with a preference for potato and rose respectively, while there was no significant preference for either substrate in the potato | rose treatment (P = 0.2145).

Furthermore, Fisher’s exact test was conducted on 2×3 contingency tables (total number of spider mites showing a preference for each substrate, per treatment, per day) to assess whether there was a significant temporal change in preference for each treatment. The results suggest any fluctuations in preference for the lemon | potato (P = 0.1743) and lemon | rose (P = 0.4394) treatments were insignificant, while significant results were obtained for the potato | rose treatment (P = 0.0294), with the overall preference shifting from potato to rose over the three days of experimentations.

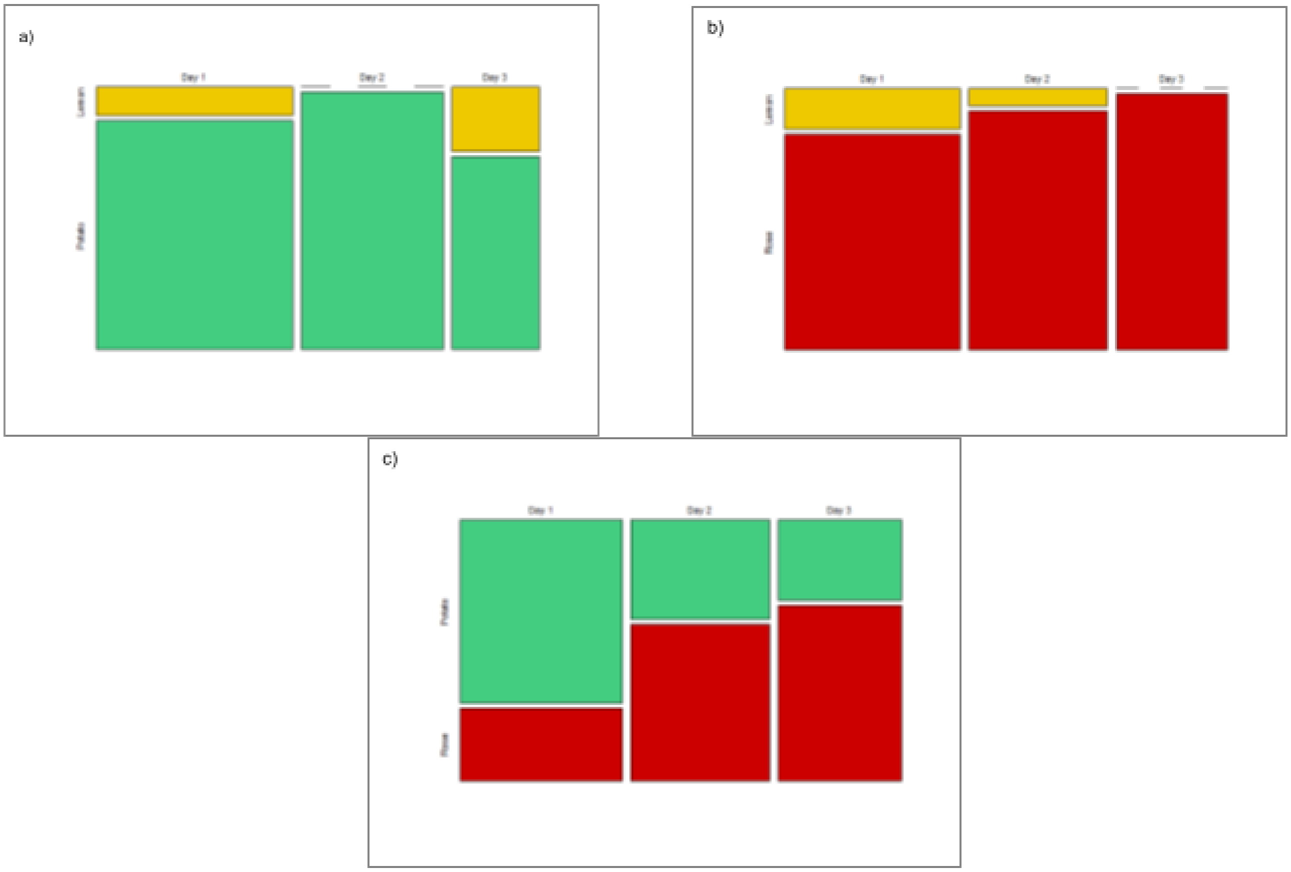

Figure 3. *Changes in relative preference over time.*
The width of each bar is indicative of the number of mites that have shown a preference for that specific day. Mosaic plots for each treatment showing the change in preference over time for:

a) lemon | potato.
b) lemon | rose.
c) potato | rose.

(Key: yellow = lemon, green = potato, red = rose)

Not all spider mites exhibited preference. **Figure 3** displays how the number of spider mites who exhibited preference varied per treatment, per day.

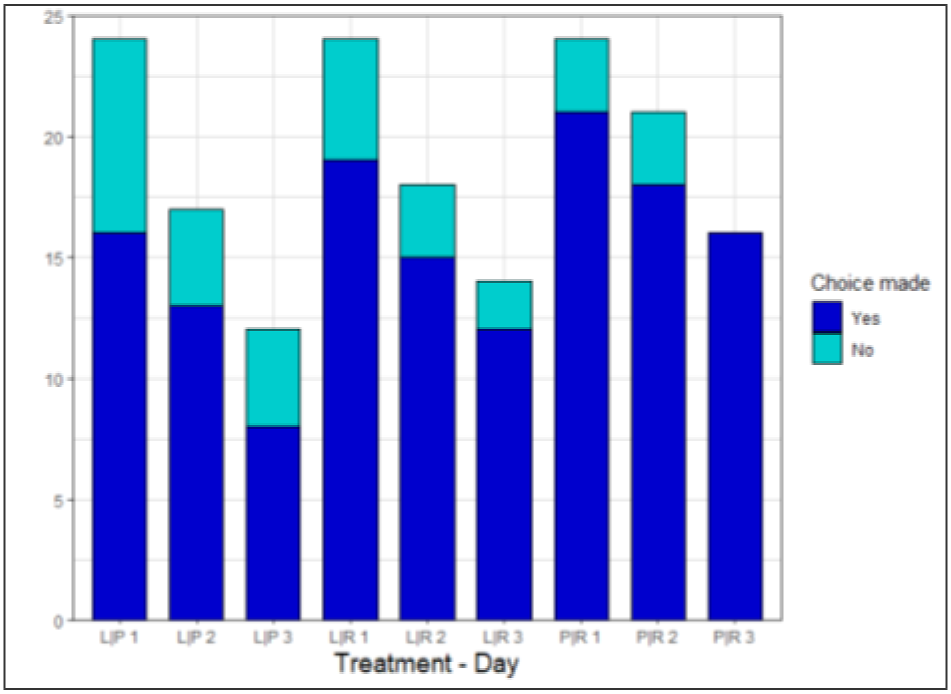

Figure 4 *Count data of mites making a choice.*
The bars are sorted in temporal order, per treatment.

Preference or aversion?

The spider mites participating in the experiment of day 4 were presented with a plant they were previously exposed to, and had displayed a preference or non-preference towards in the first 3 days of the experiment, as well as a new plant which they had never encountered before in their lives.

Thus, the mites were grouped into two treatments: mites presented with a plant they avoided on day 3 and a new plant, and mites presented with a plant they selected on day 3 and a new plant. A 2×2 contingency table (**Table 2**) was created and a Fisher's exact test was carried out. The odds ratio obtained was 1.937, with a P-value of 0.6656. The highly insignificant P-value obtained suggests that there was no significant difference between the effects of preference learning and aversion learning of host plant choice.

Day 4 preference	Familiar plant	
	Preferred plant	Non-preferred plant
Familiar plant	8	5
New plant	4	5

Table 2. *2×2 contingency table.*

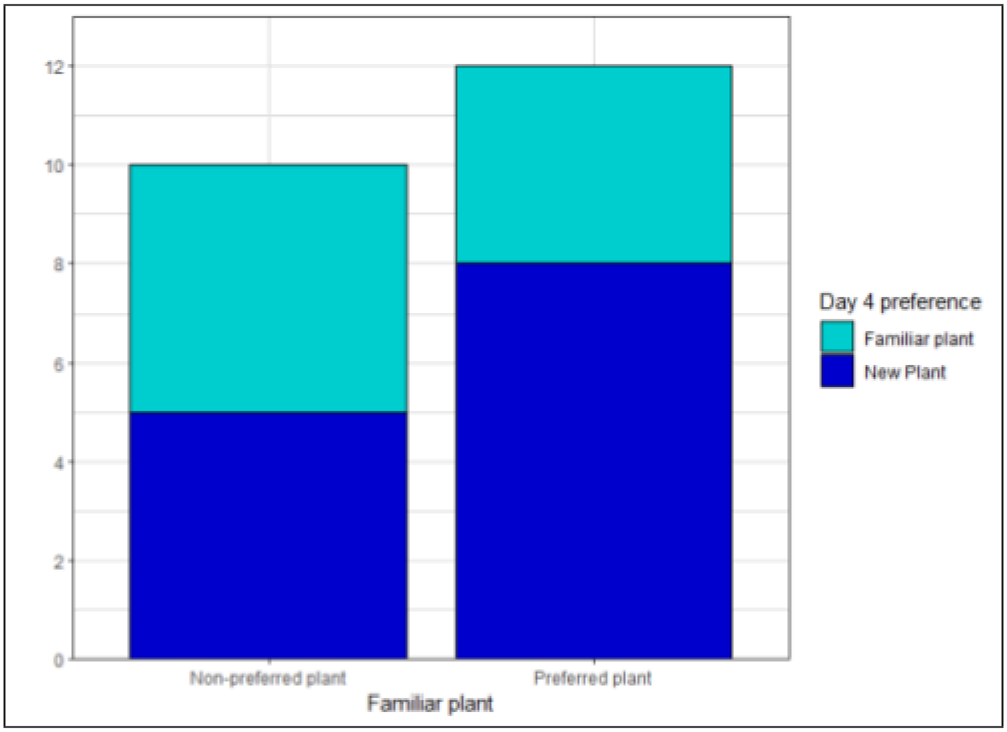

Figure 5 *Day 4 results.*
A graphical representation of the data in **Table 2**.

DISCUSSION

In an attempt to tackle answering the research question, three experiments were designed and executed, investigating: [a] the reproductive performance associated with three different oviposition and feeding substrates (lemon, potato, rose), comparing them to a control (bean), [b] the induction of preference of host plant choice, [c] the extent to which preference and aversion learning impact host plant choice induction.

The results of the first experiment suggested that the only host plant that would significantly affect the fitness of the spider mites was the lemon tree, as it was the substrate displaying the lowest total oviposition, as well as the lowest hatch rate of eggs, with post-hoc analysis indicating that it was the only substrate with a substrate significantly lower than the others. Both potato and rose did not show significant differences in their effects on fitness when compared to each other, or when compared to the control group. However, this lack of significant implications for fitness between substrates is not definitive, and thus should not be dismissed. The lack of developmental synchronization amongst the test subjects (mother mites were randomly selected from the stock population), in combination with the small sample size, could obscure any significant differences present between the host plants. Furthermore, our selection of plants was guided by the three plant defense mechanism categories (antixenosis, antibiosis, tolerance), leading to the expectation that a gradient of fitness implications should be present amongst the three plants. In the case where future research utilizing a higher number of developmentally synchronized replicants proved the absence of this expected gradient, a study similar to that conducted by Fellous et al. (2014), investigating the evolution of host range in spider mites reared on bean leaves could take place.

The results of the second experiment imply that the spider mites do indeed have preferences for certain host plants. Their choices also appear to be associated with an increase in fitness, as per the first experiment, confirming the assumption that spider mites are capable of adaptive learning, as indicated by previous research (Egas & Sabelis, 2001, Egas et al., 2003, Rovenská et al., 2005). However, this preference did not appear to be induced, but rather innate, as the fluctuations in preference over time were only significant for the spider mites which had to select between potato and rose. Future research attempting to repeat this experiment should utilize a higher number of replications, and the spider mites being used should be developmentally synchronized, as the malfunction of the incubator most likely interfered with their rate of development.

The results of the third experiment indicate that the differences in influence that preference and aversion learning have on host plant choice are highly insignificant. This conclusion however appears to lack nuance, and it is likely that the interaction between these two types of learning is complex, and circumstantial. If the results of the second experiment were to be used as a means of adding context to this claim, it would appear that both preference and aversion help the spider mites select a host plant, but to different extents based on the host plants available. The main mechanism underlying host plant choice whenever lemon was available appears to be aversion, as the results indicated that a very highly significant distaste for it was present.

However, though not statistically significant, whenever rose was available it appears that a temporal shift in preference towards it seemed to be present, implying that perhaps they develop a likeness for it. More research with more replicants , and perhaps even more host plant choices, is necessary for the latter of these two statements to be definitive.

A limitation of the third experiment is the way in which the data was analyzed. Due to the very high number of mortalities, and the already small initial sample size, the statistical analysis did not take the host plants themselves into account, and instead only looked at how likely they were to shift towards a new plant. This completely eliminates the aforementioned context necessary for the acquisition of a deeper understanding of the mechanisms behind choice.

ACKNOWLEDGEMENTS

We would like to give special thanks to our supervisor, Martijn Egas, for his support, as well as helping guide us throughout the entirety of this project. We would also like to thank Koen Freerks for sharing tips and advice with us.

REFERENCES

Agut, B., Gamir, J., Miret, J. a. J., & Flors, V. (2015). *Tetranychus urticae*-triggered responses promote genotype-dependent conspecific repellence or attractiveness in citrus. *New Phytologist (Print)*, *207*(3), 790–804. https://doi.org/10.1111/nph.13357

Altesor, P., García, Á., Font, E., Rodríguez-Haralambides, A., Vilaró, F., Oesterheld, M., Soler, R., & González, A. (2014). Glycoalkaloids of wild and cultivated solanum: Effects on specialist and generalist insect herbivores. *Journal of Chemical Ecology*, *40*(6), 599–608. https://doi.org/10.1007/s10886-014-0447-8

Awmack, C. S., & Leather, S. R. (2002). Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology (Print)*, *47*(1), 817–844. https://doi.org/10.1146/annurev.ento.47.091201.145300

Barron, A. B., Hebets, E. A., Cleland, T. A., Fitzpatrick, C. L., Hauber, M. E., & Stevens, J. R. (2015). Embracing multiple definitions of learning. *Trends in Neurosciences*, *38*(7), 405–407. https://doi.org/10.1016/j.tins.2015.04.008

Capinera, J.L. (2008). Twospotted Spider Mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). In: Capinera, J.L. (eds) Encyclopedia of Entomology. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6359-6_2602

Clotuche, G., Mailleux, A., Fernández, A. A., Deneubourg, J., Detrain, C., & Hance, T. (2011). The Formation of Collective Silk Balls in the Spider Mite *Tetranychus urticae* Koch. *PLOS ONE*, *6*(4), e18854. https://doi.org/10.1371/journal.pone.0018854

Egas, M., Norde, D., & Sabelis, M. W. (2003). Adaptive learning in arthropods: spider mites learn to distinguish food quality. *Experimental and Applied Acarology*, *30*(4), 233–247. https://doi.org/10.1023/b:appa.0000006512.26242.39

Egas, M., & Sabelis, M. W. (2001). Adaptive learning of host preference in a herbivorous arthropod. *Ecology Letters*, *4*(3), 190–195. https://doi.org/10.1046/j.1461-0248.2001.00219.x

Fellous, S., Angot, G., Orsucci, M., Migeon, A., Auger, P., Olivieri, I., & Navajas, M. (2014). Combining experimental evolution and field population assays to study the evolution of host range breadth. *Journal of Evolutionary Biology (Printed Ed.)*, *27*(5), 911–919. https://doi.org/10.1111/jeb.12362

Friedman, M. (2006). Potato glycoalkaloids and metabolites: roles in the plant and in the diet. *Journal of Agricultural and Food Chemistry*, *54*(23), 8655–8681. https://doi.org/10.1021/jf061471t

Gutierrez, J. (1985). Spider Mites: Their Biology, Natural Enemies, and Control. In W. Helle & M. W. Sabelis (Reds.), World Crop Pests: Vol. IA. Elsevier.

Hong, F., Han, H., Po, P., Wei, D., Wang, J., & Liu, Y. (2019). Effects of Five Host Plant Species on the Life History and Population Growth Parameters of *Myzus persicae* (Hemiptera: Aphididae). *Journal of Insect Science*, *19*(5). https://doi.org/10.1093/jisesa/iez094

Kavousi, A., Chi, H., Talebi, K., Bandani, A. R., Ashouri, A., & Naveh, V. H. (2009). Demographic Traits of *Tetranychus urticae* (Acari: Tetranychidae) on Leaf Discs and Whole Leaves. *Journal of Economic Entomology*, *102*(2), 595–601. https://doi.org/10.1603/029.102.0217

Macke, E., Magalhães, S., Khan, H. D., Luciano, A. A., Frantz, A., Facon, B., & Olivieri, I. (2010). Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1708), 1054–1063. https://doi.org/10.1098/rspb.2010.1706

Marinosci, C., Magalhães, S., Macke, E., Navajas, M., Carbonell, D., Devaux, C., & Olivieri, I. (2015). Effects of host plant on life‐history traits in the polyphagous spider mite *Tetranychus urticae*. *Ecology and Evolution*, *5*(15), 3151–3158. https://doi.org/10.1002/ece3.1554

Meena, N. K., Rampal, Barman, D., & Medhi, R. P. (2013). Biology and seasonal abundance of the two-spotted spider mite, *Tetranychus urticae*, on orchids and rose. *Phytoparasitica*, *41*(5), 597–609. https://doi.org/10.1007/s12600-013-0320-2

Nieberding, C. M., Van Dyck, H., & Chittka, L. (2018). Adaptive learning in non-social insects: from theory to field work, and back. *Current Opinion in Insect Science*, *27*, 75–81. https://doi.org/10.1016/j.cois.2018.03.008

Ojala, K., Julkunen-Tiitto, R., Lindström, L., & Mappes, J. (2005). Diet affects the immune defence and life-history traits of an Arctiid moth *Parasemia plantaginis*. *Evolutionary Ecology Research*, *7*(8), 1153–1170.

Papaj, D. R., & Prokopy, R. J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology*, *34*(1), 315–350. https://doi.org/10.1146/annurev.en.34.010189.001531

Rioja, C., Zhurov, V., Bruinsma, K., Grbić, M., & Grbić, V. (2017). Plant-Herbivore Interactions: A Case of an Extreme Generalist, the Two-Spotted Spider Mite *Tetranychus urticae*. *Molecular Plant-microbe Interactions*, *30*(12), 935–945. https://doi.org/10.1094/mpmi-07-17-0168-cr

Rovenská, G. Z., Zemek, R., Schmidt, J. E. U., & Hilbeck, A. (2005). Altered host plant preference of *Tetranychus urticae* and prey preference of its predator *Phytoseiulus persimilis*

(Acari: Tetranychidae, Phytoseiidae) on transgenic Cry3Bb-eggplants. *Biological Control*, *33*(3), 293–300. https://doi.org/10.1016/j.biocontrol.2005.03.017

Santamaría, M. E., Arnáiz, A., Rosa-Diaz, I., González-Melendi, P., Romero-Hernandez, G., Ojeda-Martinez, D., García, A., Contreras, E., Martínez, M., & Díaz, I. (2020). Plant Defenses Against *Tetranychus urticae*: Mind the Gaps. *Plants*, *9*(4), 464. https://doi.org/10.3390/plants9040464

Suzuki, T., España, M. U., Nunes, M. A., Zhurov, V., Dermauw, W., Osakabe, M., Van Leeuwen, T., Grbić, M., & Grbić, V. (2017b). Protocols for the delivery of small molecules to the two-spotted spider mite, *Tetranychus urticae*. *PloS One*, *12*(7), e0180658. https://doi.org/10.1371/journal.pone.0180658

Van Leeuwen, T., Tirry, L., Yamamoto, A., Nauen, R., & Dermauw, W. (2015). The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. *Pesticide Biochemistry and Physiology*, *121*, 12–21. https://doi.org/10.1016/j.pestbp.2014.12.009

APPENDIX

Attached as a PDF file.