Adaptive learning and reproductive success in female two-spotted spider mites; does mother know best?

by

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Abstract

This study investigates the ability for the two-spotted spider mite (*Tetranychus urticae*) to learn and prefer or averse one host plant above the other. *T. urticae* is a well studied herbivorous arthropod known to be able to feed and survive on hundreds of plant species due to its genetic resistance against many toxic chemicals present in the leaves of the plants. Rose, potato and lemon are being used to test their preference, with bean as a control group. The results show a statistically significant shift in preference from potato to rose, indicating the ability to learn their preferred substrate to increase their fitness. However, there was no statistically significant result in the preference versus aversion experiment which suggests that the mites do not prefer nor averse the host plants during their choice. Furthermore, experiments based on the performance of the spider mite showed that lemon was significantly less favorable than rose, potato or bean, which all three had statistically the same positive results. Overall, this study gives an insight in the adaptive learning of the two-spotted spider mite and preference versus aversion on different host plants.

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Introduction

The relationship between herbivorous arthropods and their host plant is complex and driven by multiple factors including nutritional quality and plant defenses (Marinosci et al., 2015). These factors influence the arthropod's preference for one host plant above the others and has an significant impact on the herbivore's performance, which can be expressed in reproductive success (Egas & Sabelis, 2001). The female herbivore is expected to choose the preferred host plant for oviposition to increase her offspring's fitness (Sabelis, 1991). If arthropods show adaptation in their host plant preference towards prior experience, the relationship between preference and performance should be based on the idea of learning (Egas & Sabelis, 2001).

One of these arthropods is the two-spotted spider mite (*Tetranychus urticae*), which is part of a family with more than 1200 species (Santamaria et al., 2020). The spider mite feeds on the leaves of the plant and produces a silk-like web that functions as a protection barrier and as nesting for reproduction (Santamaria et al., 2020). Female adult spider mites can lay up to ten eggs a day which grow to adulthood in about 14 days (Attia et al., 2013). This quick lifecycle makes the spider mite a perfect herbivorous arthropod to study.

Spider mites are known to be able to feed and survive on hundreds of plant species (Egas & Sabelis, 2001). This broad pallet is partly possible due to the genetic resistance against many toxic chemicals present in the leaves of the plants (Santamaria et al., 2020). These toxic chemicals are a defense mechanism adapted by the plants to inhibit being eaten (Bennett et al., 1994). An example is the potato leaf, which is a solanaceous plant (Friedman et al., 2006). These leaves contain steroidal alkaloids, which are secondary metabolites (Friedman et al., 2006). Solanine is the most abundant and can be detrimental for many herbivorous arthropods (Altesor et al, 2014). Other plants are less lucky and do not contain enough of these inherited defense mechanisms to inhibit being eaten.

Given that the two-spotted spider mite can choose between hundreds of host plants, there should be another explanation besides their genetic preference to select a preferred host. This shows that there could be adaptive learning by the arthropod, indicated by Egas & Sabelis (2001). Adaptive learning can be tested by monitoring if behavior changes with experience such that reproductive performance is improved (Egas & Sabelis, 2001). This adaptive learning of the two-spotted spider mite should increase its fitness by choosing the plant species which is the best feeding choice (e.g. no or less toxic compounds compared to another choice) (Egas et al., 2003). This also raises the question if the spider mite learns to prefer one host or learns to dislike another host.

For this research potato, rose and lemon are being used as host plants to research the ability to learn of the two-spotted spider mite. This report will address the critical question: does adaptive learning promote preference or does it promote aversion towards a specific substrate?

Materials and methods

Mites and plants

The two-spotted spider mite *Tetranychus urticae* is a polyphagous, herbivorous chelicerate with a life cycle of 8-17 days (Capinera, 2008). Adults 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). 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., 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.

For this experiment, the mites are collected from the greenhouse of the University of Amsterdam. The selection of plant species for experimentation was guided by the three categories characterizing arthropod-plant interactions: deterrence, resistance, and tolerance (Santamaria et al., 2020). Deterrence refers to mechanisms by which arthropods avoid plant hosts through external cues such as color or odors. Resistance indicates active defense mechanisms exhibited by plants against arthropod herbivory, while tolerance represents a plant's capacity to endure and recover from potential damage inflicted by arthropod feeding (Santamaria et al., 2020).

In this study, bean (*Phaseolus vulgaris)* serves as the reference plant due to the already known interaction with *T. urticae*. Rose (*Rosa spp.*) is chosen as the tolerant plant species, due to its observed adaptive responses to herbivory in past research (Meena et al., 2013). Potato plant *(Solanum tuberosum)* and lemon tree (*Citrus × Limon)* are both regarded as hostile hosts. The potato is selected for its production of secondary metabolites known to harm arthropod herbivory, while the lemon also displays differential resistance against arthropods (Agut et al., 2015).

All the plants were collected from climate-controlled greenhouses prior to the experiment. At the start of the experiment the plants were moved to the lab, where they only got water for the remaining days.

Preparation of spider mites populations

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 preference experiment was to acquire 150 female 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 mite would lay ~5 eggs per day (Capinera, 2008). The spider mites were then placed on *P. vulgaris* 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.

The fitness experiment aimed towards investigating oviposition and hatch rate on different substrates (*C. limon*, *S. tuberosum*, *Rosa spp.*), as well as *P. vulgaris* as a control, as proxies for fitness. 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 4 or 5 mites.

A protocol devised by Suzuki et al. (2017) was used as a basis for the formulation of our own protocol for the handling 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 press down to make them as flat as possible.
5. Transfer adult female mites to the leaf disks from the stock population using a fine paintbrush to pick them up.
6. Incubate the mites for 48 hours at 23°C, with 16 hours of light exposure and 8 hours of dark 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 ensure everything is fine.
10. In the case that the leaf disks desiccate, add additional leaf disks to provide any hatched larvae with an adequate source of nutrition.

Experimental setup

To test the performance, which is used to determine fitness, the mites on the used substrates are tested by starting multiple egg waves on the different substrates, two different experimental setups were planned, with an experimental mite population prepared for each setup. The two populations were prepared using different techniques to accommodate to the requirements of each experiment, while simultaneously ensuring that all individuals within each population were developmentally synchronized.

Choice arenas

To understand if the mites develop an avoidance or prefer to a certain host they first have to undergo learning, this is done by utilizing a choice experiment which is done by using multiple numbered arenas placed in a protective open box. These choice arenas are made up of 2 different halve discs placed together to form a full disc. The halves are made of leaf discs with a diameter of 10 millimeters, consisting of different plants, in this case either potato, rose or lemon. The choice halves are connected with a glass headed pin in the middle.

The three different arena combinations are: potato - rose; potato - lemon; rose - lemon. To keep the leaves and pin in place, and prevent them from desiccating, the arenas are put on a layer of moist cotton wool. Each arena possesses one female mite from eggwave one, or from the second batch which was done due to time constraints and low female concentrations of eggwave one, these females are transferred onto the pin using a small paintbrush. Once the females are on the pin, they are left alone for 24 hours, in which they tend to crawl down the pin onto the leaf disc, may essay both halves where after they establish themselves. When established they start producing webbing, thus staying in relatively the same position (Gutierrez, 1985), and start to lay eggs when enough nutrients are consumed.

To keep the mites undisturbed and at a more ideal climate, the the arenas are put in the previously mentioned climate controlled incubator at 23*C during the given 24 hours. After each 24 hours the mites are scored on what their position is, by how many eggs are present and on which plant, when eggs are present on both leaf types the side with most eggs is considered as preference. Next the mite is transferred to a new fresh arena which is identical to the one it was put on for the first day, with the same number to keep track of oviposition per mite. This transferring is done on day 2 and day 3 of the experiment. Whenever the mites don't survive the 24 hours to the next day, they are excluded from further analysis.

To test for if the mites start to develop a preference for the one plant or a distaste for the other plant, day 4 is used. This day is set up by having the mites from day 3 and transferring half the mites per combination group to new arenas, which are built up the same as for the other days, consisting of one overlapping plant from the first arena combined with the not used plant. With

the other half of the group going on new arenas built up of the other original arena plant with the not used plant, for example the mites from rose - potato arenas, half of them go to potato - lemon while the other half goes to rose - lemon arenas.

Results

Preference experiment

In the initial phase of the preference experiment, the mites were presented with a choice between two host plants. Three choice arena's were made consisting of: potato/lemon, rose/potato and lemon/rose. To test the change in host plant preference, Fisher's exact test for count data was used per (Upton, 1992).

For the [potato/lemon] experiment (Fig. 1), the mites showed a preference for potato over lemon (p-value = 3.353161e-7). The p-value of 0.1837 indicates that no statistically significant change in preference between the two host plants was detected. Therefore, it can be concluded that the mites did not demonstrate a significant shift in their preference for host plants over the duration of the initial experiment.

For the [rose/potato] experiment (Fig. 2), the mites showed no preference between potato and rose (p-value = 0.2145392). The p-value of 0.02942 indicates that there is a statistically significant change in preference between the two host plants. Therefore, it can be concluded that the mites did demonstrate a significant shift in their preference for host plants over the duration of the initial experiment.

For the [lemon/rose] experiment, the mites showed a preference for rose over lemon (p-value = 5.10019e-09). The p-value of 0.4394 indicates that no statistically significant change in preference between the two host plants was detected. Therefore, it can be concluded that the mites did not demonstrate a significant shift in their preference for host plants over the duration of the initial experiment.

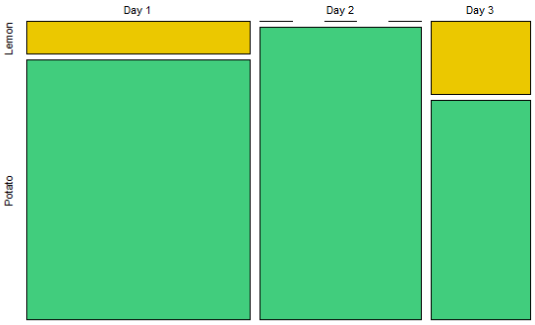

Figure 1 Fraction of mites choosing between potato or lemon on three consecutive days.

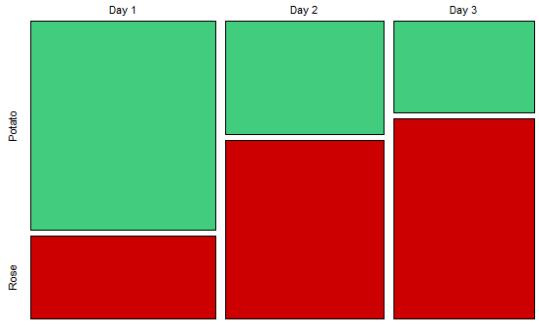

Figure 2 Fraction of mites choosing between rose or potato on three consecutive days.

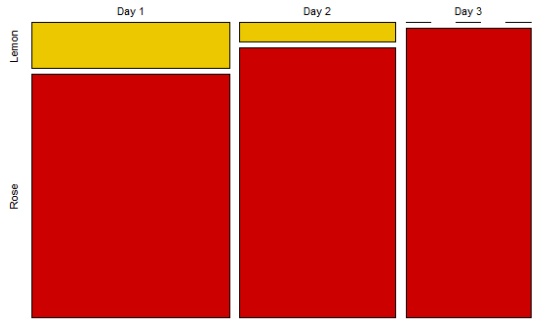

Figure 3 Fraction of mites choosing between rose or lemon on three consecutive days.

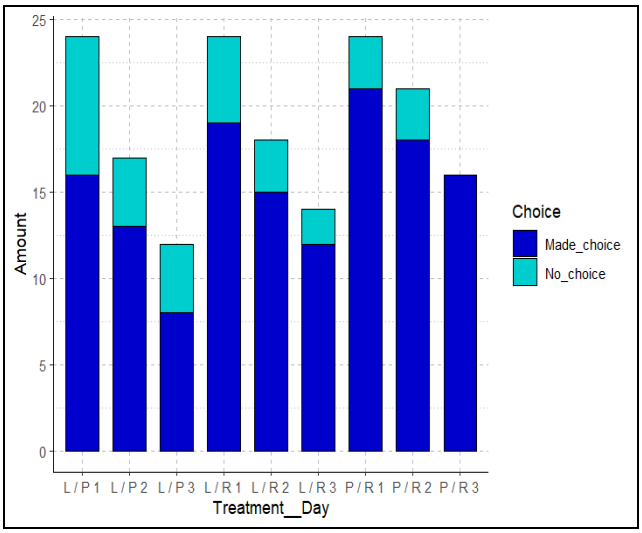

Figure 4 Amount of mites per treatment per day that made a choice

In addition, a graph (Fig. 4) showing the amount of mites per treatment per day that made a choice between host plants and are therefore successfully transferred to the subsequent day of the experiment. Because the results in the previous shown mosaic plots are given in fractions. The amount of mites decreased throughout the experiment resulting in fewer mites for day 4.

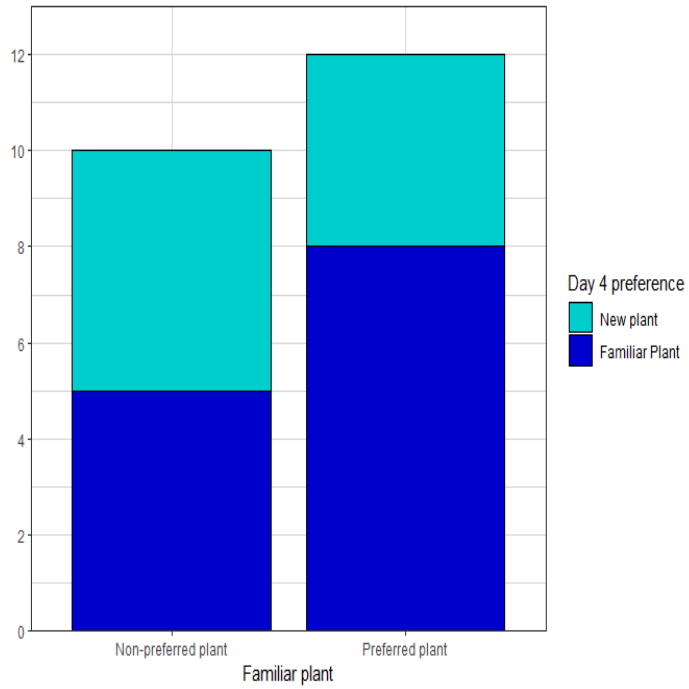

Figure 5 Fraction of mites choosing between familiar plant or new plant

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

Preference versus aversion

The 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 (Fig. 5). A contingency table (table 1) 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 low odds ratio and highly non-significant P-value obtained suggest that there was no significant difference between the effects of preference and aversion of host plant choice.

Table 1 Fraction of mites choosing between familiar plant or new plant

Performance

For the control (bean), both the tested fitness measures were the highest, as it had the largest amount of eggs with 42, and the largest hatch rate at 0.976 by day 10.

Of the other tested hosts the lemon plant had both the lowest oviposition and hatch rate, with only 1 hatched out of 14 eggs resulting in a rate of 0.071.

The rose had the second highest hatch rate (0.769), however it had the second lowest oviposition at 26 eggs, as the potato had 28 eggs, with a hatch rate of 0.607.

Using a repeated measure ANOVA on the (arcsine root) transformed hatch rate per day, resulted in a P-value of 8.38e-09. Which implies a significant difference between the different substrates.

To determine which combinations were significantly different, Tukey's HSD (Honestly Significant Difference) test, with the “Bonferroni” adjustment method was utilized.

This indicates that only the lemon is significantly different from all other hosts in hatch rate, there was no significant difference between the other hosts.

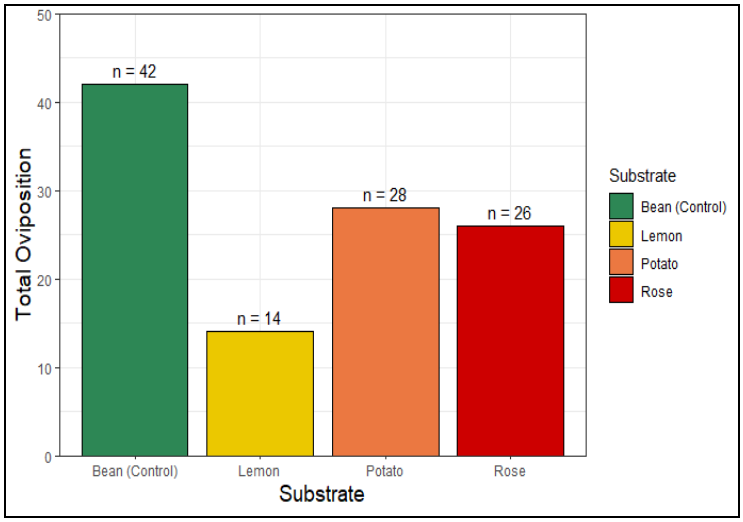

Figure 6 Total oviposition on the four substrates

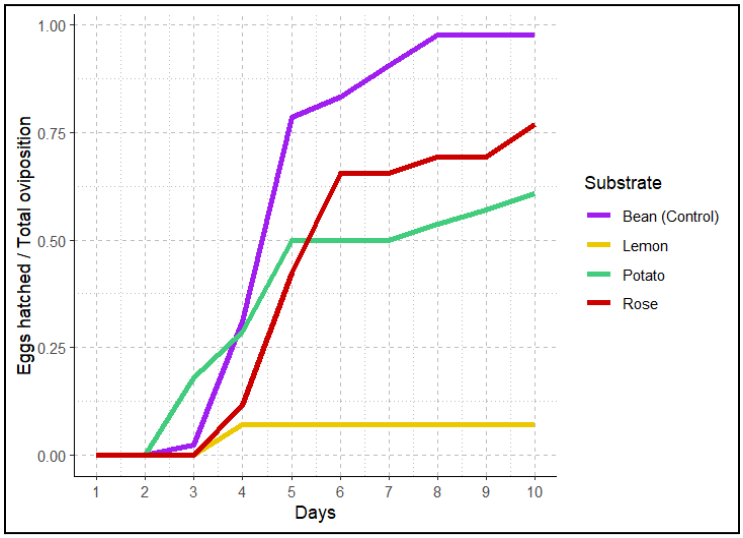

Figure 7 Total oviposition per substrate per day

Group1 - Group 2	P-value
1 Fraction_transformed Bean - Lemon	0.00405
2 Fraction_transformed Bean - Potato	0.471
3 Fraction_transformed Lemon - Potato	0.0248
4 Fraction_transformed Bean - Rose	0.395
5 Fraction_transformed Lemon - Rose	0.0336
6 Fraction_transformed Potato - Rose	0.895

Table 2 p-values between substrates total oviposition

Discussion

To conclude, the preference experiment showed statistically significant difference in the [rose/potato] trial where the preference switched from potato to rose. The low p-values of [rose/lemon] and the [lemon/potato] trials did show a significant preference for one substrate over the other, but no significant difference in the change of preference with p-values 0.4394 and 0.1837.

Furthermore, the preference versus aversion experiment, which followed the preference experiment as ‘Day 4’, also showed no statistically significance (p-value: 0.6656), which suggest that the mites do not prefer nor averse the host plants during their choice. An additional aspect to consider is that the mites did avoid lemon in almost all cases but were drawn towards rose, with potato being the intermediate choice. The performance test showed that lemon performed noticeably worse than bean, rose or potato, with all the p-values being lower than 0.05 for the combinations with lemon.

These results show that mites will avoid negatively associated host plants, and in abscess can develop a preference for the better host. This suggests that the ability of adaptive learning is present in mites to increase the fitness of the next generation, which corresponds with previous findings by Egas & Sabelis (2001).

However, it is important to acknowledge the limitations in significant results of this experiment. The preference experiment showed only a 33% significance rate whereas the preference versus aversion experiment had no significance at all. The performance experiment showed similar results for potato, bean and rose and a strong dislike for lemon. Spider mites have a strong aversion against citrus, which correlates with the research by Agut et al (2015).

A possible explanation for the limitations is a small sample size, the expected amount of mites differed a lot from the actual amount of mites being used. This happened due to multiple circumstances, one of them is that the eggwave did not end up as expected; the optimal incubator conditions, given by Helle & Sabelis (1985), were different and thereby misinterpreted. Additionally, due to a technical error occurring in the incubator where the mites were kept, led to 4 days without any light (whereas normal setting refers to 16 hours of light per day) and a decrease in temperature from 23 degrees to 20 degrees. Next to that, during the preference and preference versus aversion experiments there were high mortality rates, resulting in less and less mites to work with.

Another issue that arose during the experiment was the shrinking of potato leaves during the preference versus aversion experiments which resulted in mites ending up in the cotton wool.

In conclusion, the research shows promising findings on the adaptive learning of spider mites, but further research should focus on the preference versus aversion experiment. While the idea was promising, the experiment itself showed no significant results due to high mortality rates.

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Appendix

Available on the next pages.

Day 1								Day 2								Day 3	
Batch	Treatment	ID	Oviposition		Position	Preference	Alive (A) | Dead (D)	Batch	Treatment	ID	Oviposition		Position	Preference	Alive (A) | Dead (D)	Batch	Treatment
			Plant 1	Plant 2							Plant 1	Plant 2					
OGs	L | P	A	0	2	Potato	Potato	A	OGs	L | P	A	0	0	Potato	Potato	A	OGs	L | P
OGs	L | P	B	0	1	Potato	Potato	A	OGs	L | P	B	0	0	NA	No preference	D		
OGs	L | P	C	0	2	Potato	Potato	A	OGs	L | P	C	0	0	NA	No preference	D		
OGs	L | P	D	0	1	Potato	Potato	A	OGs	L | P	D	0	0	Potato	Potato	A	OGs	L | P
OGs	L | R	A	2	0	Lemon	Lemon	D											
OGs	L | R	B	0	0	NA	No preference	D											
OGs	L | R	C	0	0	Rose	Rose	A	OGs	L | R	C	0	0	NA	No preference	D		
OGs	L | R	D	0	1	Rose	Rose	A	OGs	L | R	D	0	3	Rose	Rose	A	OGs	L | R
OGs	P | R	A	1	2	Rose	Rose	A	OGs	P | R	A	0	0	Rose	Rose	A	OGs	P | R
OGs	P | R	B	2	1	Potato	Potato	A	OGs	P | R	B	0	0	NA	No preference	D		
OGs	P | R	C	2	0	Potato	Potato	A	OGs	P | R	C	0	0	Potato	Potato	D		
OGs	P | R	D	1	1	Rose	Rose	A	OGs	P | R	D	2	0	Potato	Potato	A	OGs	P | R
MPs	L | P	1A	0	2	NA	Potato	D											
MPs	L | P	1B	0	0	NA	No preference	D											
MPs	L | P	1C	0	0	Potato	Potato	A	MPs	L | P	1C	0	5	Potato	Potato	A	MPs	L | P
MPs	L | P	1D	0	0	NA	No preference	A	MPs	L | P	1D	0	0	Potato	Potato	A	MPs	L | P
MPs	L | P	1E	0	0	Potato	Potato	A	MPs	L | P	1E	0	4	Potato	Potato	D		
MPs	L | P	2A	0	0	NA	No preference	D											
MPs	L | P	2B	0	0	NA	No preference	D											
MPs	L | P	2C	0	1	Potato	Potato	A	MPs	L | P	2C	0	1	Potato	Potato	A	MPs	L | P
MPs	L | P	2D	0	0	Potato	Potato	A	MPs	L | P	2D	0	0	NA	No preference	D		
MPs	L | P	2E	0	0	Lemon	Lemon	A	MPs	L | P	2E	0	2	Potato	Potato	A	MPs	L | P
MPs	L | P	3A	0	0	Potato	Potato	A	MPs	L | P	3A	0	0	Potato	Potato	A	MPs	L | P
MPs	L | P	3B	1	2	Potato	Potato	A	MPs	L | P	3B	0	1	Potato	Potato	A	MPs	L | P
MPs	L | P	3C	0	1	Potato	Potato	A	MPs	L | P	3C	0	0	Potato	Potato	A	MPs	L | P
MPs	L | P	3D	0	1	Potato	Potato	A	MPs	L | P	3D	0	1	Potato	Potato	A	MPs	L | P
MPs	L | P	3E	0	0	Lemon	Lemon	A	MPs	L | P	3E	0	0	NA	No preference	D		
MPs	L | P	4A	0	0	Potato	Potato	A	MPs	L | P	4A	0	5	Potato	Potato	A	MPs	L | P
MPs	L | P	4B	0	0	NA	No preference	D											
MPs	L | P	4C	0	0	Potato	Potato	D											
MPs	L | P	4D	0	0	Potato	Potato	A	MPs	L | P	4D	0	1	Potato	Potato	A	MPs	L | P
MPs	L | P	4E	0	0	NA	No preference	D											
MPs	L | R	1A	0	0	Rose	Rose	A	MPs	L | R	1A	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	1B	0	0	NA	No preference	A	MPs	L | R	1B	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	1C	0	0	NA	No preference	D											
MPs	L | R	1D	0	1	Rose	Rose	A	MPs	L | R	1D	0	3	Rose	Rose	A	MPs	L | R
MPs	L | R	1E	0	3	Rose	Rose	A	MPs	L | R	1E	0	5	Rose	Rose	A	MPs	L | R
MPs	L | R	2A	0	4	Rose	Rose	A	MPs	L | R	2A	0	5	Rose	Rose	A	MPs	L | R
MPs	L | R	2B	0	3	NA	Rose	D											
MPs	L | R	2C	1	2	Rose	Rose	A	MPs	L | R	2C	0	1	Rose	Rose	A	MPs	L | R
MPs	L | R	2D	0	2	Rose	Rose	A	MPs	L | R	2D	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	2E	0	0	Lemon	Lemon	A	MPs	L | R	2E	0	0	NA	No preference	D		
MPs	L | R	3A	0	0	NA	No preference	D											
MPs	L | R	3B	0	0	Rose	Rose	A	MPs	L | R	3B	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	3C	0	0	NA	No preference	D											
MPs	L | R	3D	2	1	Lemon	Lemon	A	MPs	L | R	3D	1	0	Lemon	Lemon	A	MPs	L | R
MPs	L | R	3E	0	0	Rose	Rose	A	MPs	L | R	3E	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	4A	0	0	Rose	Rose	A	MPs	L | R	4A	0	0	Rose	Rose	D		
MPs	L | R	4B	0	3	Rose	Rose	A	MPs	L | R	4B	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	4C	0	0	Rose	Rose	A	MPs	L | R	4C	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	4D	0	4	Rose	Rose	A	MPs	L | R	4D	0	0	Rose	Rose	A	MPs	L | R
MPs	L | R	4E	0	6	Rose	Rose	A	MPs	L | R	4E	0	0	NA	No preference	D		
MPs	P | R	1A	6	0	Potato	Potato	A	MPs	P | R	1A	0	6	Rose	Rose	A	MPs	P | R
MPs	P | R	1B	6	0	Potato	Potato	A	MPs	P | R	1B	1	3	Rose	Rose	A	MPs	P | R
MPs	P | R	1C	5	0	Potato	Potato	A	MPs	P | R	1C	0	3	Rose	Rose	A	MPs	P | R
MPs	P | R	1D	1	1	Potato	Potato	A	MPs	P | R	1D	3	0	Potato	Potato	A	MPs	P | R
MPs	P | R	1E	3	0	Potato	Potato	A	MPs	P | R	1E	3	0	Potato	Potato	A	MPs	P | R
MPs	P | R	2A	4	0	Potato	Potato	A	MPs	P | R	2A	0	4	Rose	Rose	A	MPs	P | R
MPs	P | R	2B	6	0	Potato	Potato	A	MPs	P | R	2B	2	2	Potato	Potato	A	MPs	P | R
MPs	P | R	2C	2	2	Rose	Rose	A	MPs	P | R	2C	0	0	Rose	Rose	A	MPs	P | R
MPs	P | R	2D	5	0	Potato	Potato	A	MPs	P | R	2D	0	0	NA	No preference	D		
MPs	P | R	2E	7	0	Potato	Potato	A	MPs	P | R	2E	0	0	NA	No preference	D		
MPs	P | R	3A	5	0	Potato	Potato	A	MPs	P | R	3A	0	5	Rose	Rose	A	MPs	P | R
MPs	P | R	3B	6	0	Potato	Potato	A	MPs	P | R	3B	5	0	Potato	Potato	A	MPs	P | R
MPs	P | R	3C	3	0	NA	Potato	D											
MPs	P | R	3D	4	0	Potato	Potato	A	MPs	P | R	3D	0	5	Rose	Rose	A	MPs	P | R
MPs	P | R	3E	0	0	NA	No preference	D											
MPs	P | R	4A	0	0	Rose	Rose	A	MPs	P | R	4A	0	0	Rose	Rose	A	MPs	P | R
MPs	P | R	4B	0	5	Rose	Rose	A	MPs	P | R	4B	0	0	Rose	Rose	D		
MPs	P | R	4C	1	3	Rose	Rose	A	MPs	P | R	4C	0	4	Rose	Rose	A	MPs	P | R
MPs	P | R	4D	0	0	NA	No preference	D											
MPs	P | R	4E	5	0	Potato	Potato	A	MPs	P | R	4E	3	0	Potato	Potato	A	MPs	P | R

						Day 4									
ID	Oviposition		Position	Preference	Alive (A) | Dead (D)	Batch	Treatment	ID	New Treatment	New ID	Oviposition		Position	Preference	Alive (A) | Dead
	Plant 1	Plant 2									Plant 1	Plant 2			
A	0	0	NA	No preference	D										
D	0	0	Potato	Potato	A	OGs	L | P	D	L | R	1B	0	0	Rose	Rose	A
D	0	0	Rose	Rose	D										
A	1	0	Potato	Potato	A	OGs	P | R	A	L | P	3E	0	0	Potato	Potato	A
D	1	0	NA	Potato	D										
1C	0	0	Potato	Potato	A	MPs	L | P	1C	P | R	3A	0	2	Rose	Rose	A
1D	0	0	Potato	Potato	A	MPs	L | P	1D	L | R	2A	0	0	Rose	Rose	A
2C	0	0	NA	No preference	D										
2E	0	0	NA	No preference	D										
3A	0	0	Lemon	Lemon	D										
3B	1	0	Lemon	Lemon	A	MPs	L | P	3B	L | R	1A	0	0	Rose	Rose	A
3C	0	0	NA	No preference	D										
3D	0	1	NA	Potato	D										
4A	0	2	Potato	Potato	D										
4D	0	0	Potato	Potato	A	MPs	L | P	4D	P | R	4A	0	0	NA	No preference	D
1A	0	0	NA	No preference	D										
1B	0	3	Rose	Rose	A	MPs	L | R	1B	P | R	3A	0	0	Rose	Rose	A
1D	0	5	Rose	Rose	A	MPs	L | R	1D	P | R	3B	0	0	Potato	Potato	A
1E	0	3	Rose	Rose	A	MPs	L | R	1E	P | R	3C	0	0	Rose	Rose	A
2A	0	5	Rose	Rose	A	MPs	L | R	2A	L | P	1A	0	0	Potato	Potato	A
2C	0	0	NA	No preference	D										
2D	0	0	Rose	Rose	A	MPs	L | R	2D	L | P	2B	0	0	NA	No preference	D
3B	0	2	Rose	Rose	A	MPs	L | R	3B	L | P	2A	0	0	Potato	Potato	A
3D	0	4	Rose	Rose	A	MPs	L | R	3D	P | R	4B	0	3	Rose	Rose	A
3E	0	4	Rose	Rose	A	MPs	L | R	3E	L | P	2C	0	0	Lemon	Lemon	A
4B	0	5	Rose	Rose	A	MPs	L | R	4B	P | R	4A	0	0	NA	No preference	D
4C	0	4	Rose	Rose	A	MPs	L | R	4C	L | P	1B	0	0	NA	No preference	D
4D	0	5	Rose	Rose	A	MPs	L | R	4D	L | P	1C	0	0	Lemon	Lemon	A
1A	0	5	Rose	Rose	A	MPs	P | R	1A	L | R	1A	0	3	Rose	Rose	A
1B	0	4	Rose	Rose	A	MPs	P | R	1B	L | P	4A	0	1	Potato	Potato	A
1C	0	0	Rose	Rose	A	MPs	P | R	1C	L | P	3B	0	0	Lemon	Lemon	A
1D	0	3	Rose	Rose	A	MPs	P | R	1D	L | P	3C	0	1	Potato	Potato	A
1E	0	0	Rose	Rose	A	MPs	P | R	1E	L | P	4C	0	0	Lemon	Lemon	A
2A	2	0	Potato	Potato	A	MPs	P | R	2A	L | P	3A	0	2	Potato	Potato	A
2B	2	2	Rose	Rose	A	MPs	P | R	2B	L | R	1B	0	0	Rose	Rose	A
2C	0	5	Rose	Rose	A	MPs	P | R	2C	L | P	4B	0	0	NA	No preference	D
3A	0	5	Rose	Rose	A	MPs	P | R	3A	L | R	2A	0	0	NA	No preference	D
3B	0	0	Potato	Potato	A	MPs	P | R	3B	L | R	2B	0	0	NA	No preference	D
3D	0	0	Rose	Rose	A	MPs	P | R	3D	L | R	2C	0	0	Rose	Rose	A
4A	0	0	Rose	Rose	D										
4C	1	0	Potato	Potato	A	MPs	P | R	4C	L | R	1C	0	1	Rose	Rose	A
4E	0	2	Rose	Rose	A	MPs	P | R	4E	L | R	1D	0	0	NA	No preference	D

Number of mites per treatment per day:

	Day 1	Day 2	Day 3	Day 4
L | P	24	17	12	5
L | R	24	18	14	11
P | R	24	21	16	14

Number of mites showing preference:

		Day 1	Day 2	Day 3
L | P	Lemon	2	0	2
	Potato	16	13	6
L | R	Lemon	3	1	0
	Rose	16	14	12
P | R	Potato	15	7	5
	Rose	6	11	11

Relative preference:

		Day 1	Day 2	Day 3
L | P	Lemon	0.083	0.000	0.167
	Potato	0.667	0.765	0.500
L | R	Lemon	0.125	0.056	0.000
	Rose	0.667	0.778	0.857
P | R	Potato	0.625	0.333	0.313
	Rose	0.250	0.524	0.688

Previous treatment	New Treatment	Substrate	No. of mites showing preference
L | P	L | R	Lemon	0
		Rose	3
	P | R	Potato	0
		Rose	1
L | R	L | P	Lemon	2
		Potato	2
	P | R	Potato	1
		Rose	3
P | R	L | P	Lemon	2
		Potato	4
	L | R	Lemon	0
		Rose	4

Total oviposition per day:

		Day 1	Day 2	Day 3
L | P	Lemon	1	0	1
	Potato	13	20	3
L | R	Lemon	5	1	0
	Rose	30	17	40
P | R	Potato	75	19	7
	Rose	15	32	26

Relative oviposition per day:

		Day 1	Day 2	Day 3
L | P	Lemon	0.071	0.000	0.250
	Potato	0.929	1.000	0.750
L | R	Lemon	0.143	0.056	0.000
	Rose	0.857	0.944	1.000
P | R	Potato	0.833	0.373	0.212
	Rose	0.167	0.627	0.788