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

by

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Abstract

This study investigates the ability for the two-spotted spider mite (*Tetranychus urticae*) to develop preferences or aversions for one host plant over the other. *T. urticae* is a well studied herbivorous arthropod known to be able to feed and survive on a wide range of plant species due to its genetic resistance against many toxic chemicals present in the leaves of certain plants. Rose, potato and lemon are being used to test their preference and performance, with bean as a control group only in the performance part. 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 favourable 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

Herbivorous arthropods have quite complex relationships with their host plants, which is influenced by multiple factors, of which nutritional quality and the defences of the plant (Marinosci et al., 2015). The preference of the arthropods too is influenced by these factors, which can have a strong effect on the animals performance, this can be expressed as reproductive success (Egas & Sabelis, 2001). To increase the offspring's fitness, the female tends to choose their preferred host plant for oviposition when available (Sabelis, 1991). When 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).

A perfect to study herbivorous arthropod is the two-spotted spider mite (*Tetranychus urticae*). This is due to their quick lifecycle (Naher et al., 2008), as the mites can grow up to adulthood in about 14 days (Attia et al., 2013), and high oviposition rate, each female can lay up to 10 eggs per day within a close to optimal environment (Attia et al., 2013). The two-spotted spider mite produces a silk-like web that functions as a protection barrier and as nesting for reproduction (Santamaria et al., 2020), thus the mites don't migrate much when this web is in place (Helle & Sabelis., 1985).

The spider mites are able to feed and survive on a wide range of plant species (Egas & Sabelis, 2001). This wide array is partially made possible by genetic resistances against a broad scope of toxic chemicals which are present in the leaves of certain plants (Santamaria et al., 2020). These toxins are defence mechanisms adapted by the plants to avoid being fed upon (Bennett et al., 1994). Toxins are one of three mechanisms plants developed (relative to spider mites): which are: antixenosis, antibiosis, and tolerance (Santamaría et al., 2020). Antixenosis represents a feeding aversion characterised 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). Take as an example the leaves of potatoes which contain the secondary metabolites, steroidal alkaloids (Friedman et al., 2006), which is a form of antibiosis (Santamaría et al., 2020). Or those of citrus plants, **…** which are categorised as antixenosis (Santamaría et al., 2020). However many plants like roses lack these types of defences (as roses only gain chemical defences when in symbiosis with fungi (Yang et al., 2015)) against herbivorous arthropods and are thus a prime host for mites.

As mentioned the two-spotted spider mite has a broad range of possible host plants, however they do prefer certain plants over others. This can be explained by a genetic preference, however this effect doesn't explain it completely, and with previous research indicating that these mites are capable of learning, as they adjust their behaviour when presented with different stimuli. Specifically, spider mites have been shown to be able to discriminate between host plants based on species (Egas & Sabelis, 2001). Leads the way for adaptive learning to also play a role, as indicated by Egas & Sabelis (2001). Adaptive Learning can be described as "Adaptive learning is defined as a change in behaviour as a result of previous experiences that leads to an increase in reproductive success" (Egas & Sabelis, 2001). This can be tested by monitoring if behaviour changes with experience such that reproductive performance is improved (Egas & Sabelis, 2001). Thus the 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 report will address the previously mentioned adaptive learning in the two-spotted spider mite, by monitoring the mites choices and if these are in actuality the better option to increase fitness. However the main point of interest to be discussed is: By adaptively learning do spider mites develop a preference for a certain host plant or do they develop an aversion towards other plants, when the relative fitness decreases?

Methodology

Plant choice

For this experiment, *Tetranychus urticae*, a polyphagous herbivorous mite, was selected as the subject organism, collected from the greenhouse of the University of Amsterdam. The selection of plant species for experimentation was guided by the three categories characterising 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 colour or odours. Resistance indicates active defence 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. 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.

Spider Mites

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.

Experimental setup

Preparation of spider mite populations

In order to investigate preference for specific substrates in spider mites, as well as the fitness associated with each substrate, 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 synchronised.

Female mites were collected from the aforementioned stock population, which consisted of both male and female individuals, to ensure that all females extracted were fertilised, 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 minimises 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 performance 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.
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.

Performance
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; potato, rose and lemon. A fourth eggwave for this is done using bean as a reference, this because the bean has been the substrate from which the mites were collected and thus a general survivability is known. Next, two days after termination of eggwaves the discs are observed every 24 hours for 12 days, the results scored are: The amount of eggs present, The number of these eggs that have hatched and lastly how many mites are dead.

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 utilising 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 (Helle & Sabelis., 1985), and start to lay eggs when enough nutrients are consumed (Helle & Sabelis., 1985). To keep the mites undisturbed and at a more ideal climate, for our time, as warmer climate leads to faster development(to a certain extend)(Capinera, 2008), 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.

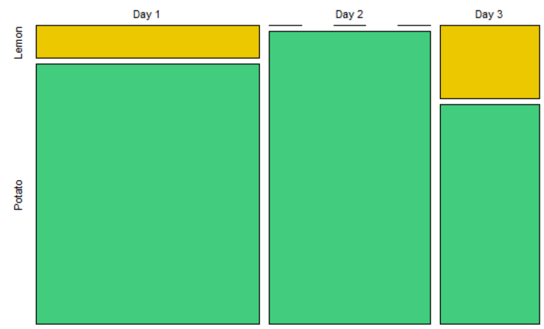

Figure 1 Fraction of mites choosing between potato or lemon on three consecutive days.

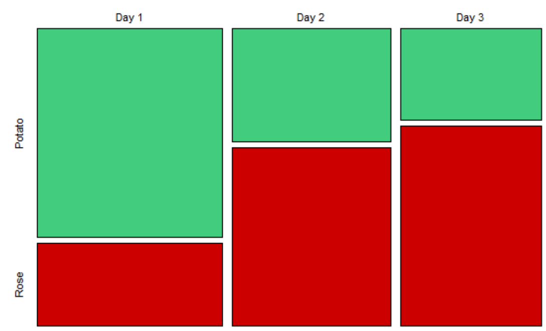

Figure 2 Fraction of mites choosing between rose or potato on three consecutive days.

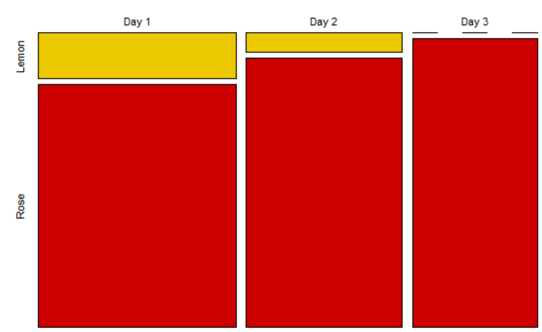

Figure 3 Fraction of mites choosing between rose or lemon on three consecutive days.

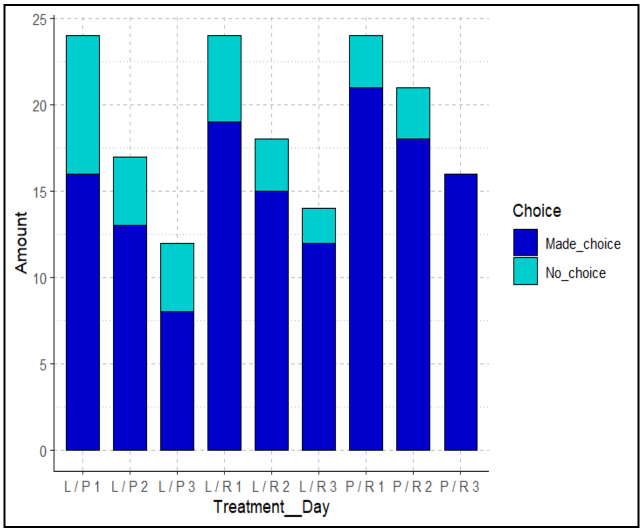

Figure 4 Amount of mites per treatment per day that made a choice

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.

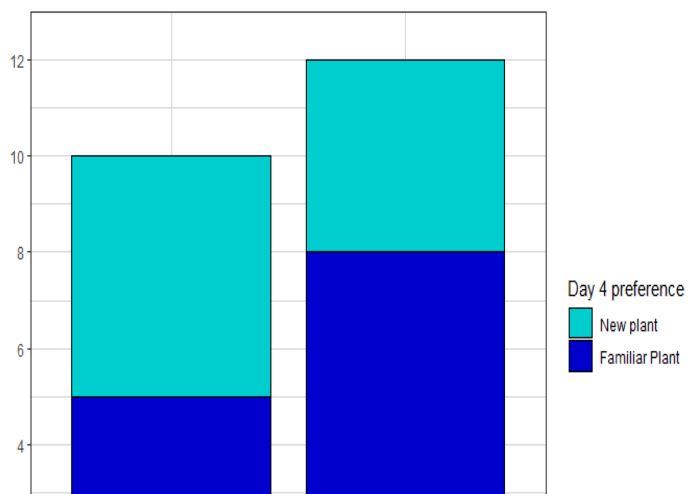

Figure 5 Fraction of mites choosing between familiar plant or new plant

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 utilised.

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.

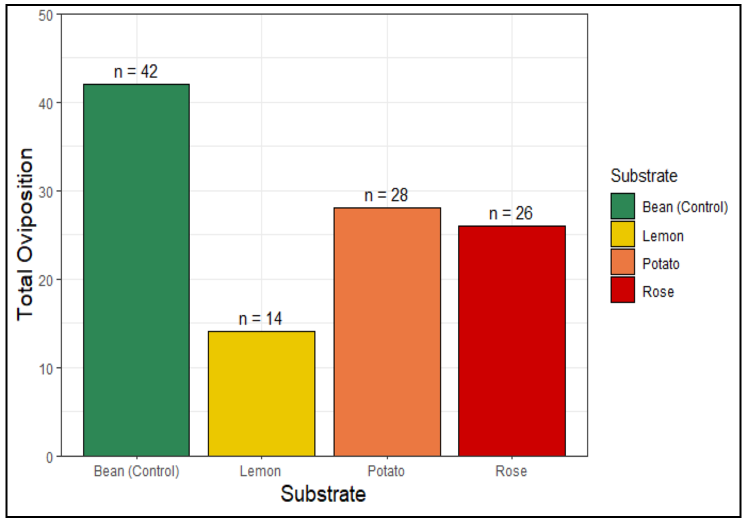

Figure 6 Total oviposition on the four substrates

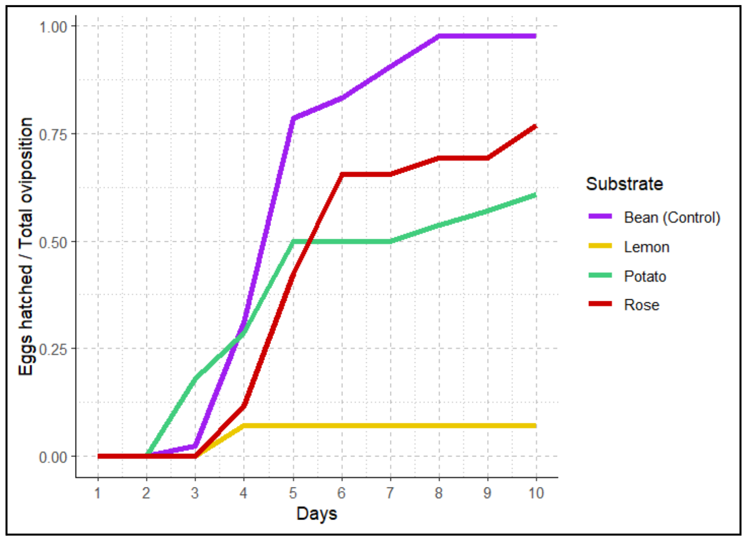

Figure 7 Total oviposition per substrate per day

Table 2 p-values between substrates total oviposition

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

Discussion

Using the acquired data the conclusion can be drawn that the mites on the rose - lemon and lemon - potato treatments don't develop a preference over the 3 days, however the rose - potato treatment did develop a preference as the fraction choosing potato moved significantly away from potato towards a higher fraction of rose.
From day 3 to 4 the tested influence of preference and aversion on host choice suggests that the mites do not show any effect of preference or aversion, which means that these equally affect the choice. When confronted with in this case lemon the mites generally avoid it when possible, but when rose is present they prefer that over the other options, with potatoes being the middle ground in this situation.

The performance test resulted in the fact that only lemon is significantly worse as a host for the spider mite when it comes to the fitness measures used, with bean, which was used as control, rose and potato being similar in performance.

These results suggest a slight learning effect in mites when substrates are relatively similar as hosts for fitness performance, but not when there is a clearly better host present.
And as day 4 indicates that preference and aversion affect the choice equally implies that when there is a clearly bad host they avoid that host, and when there is no clearly better host, they start to develop a preference for the slightly (insignificantly) better host.

However due to the extremely low sample sizes, for a behavioural experiment, these conclusions are not reliable and only valid as inspiration for further research.
These low sample sizes happened due to time constraints coupled with a string of unfortunate complications. The first of which is overestimating oviposition, as sources mentioned that the female mites lay up to 10 eggs per day (Helle & Sabelis., 1985), however this was at higher temperatures than the incubator used(23℃), in combination with possibly misidentifying adult females leading to a lower oviposition in our case. Also an error occurred in the incubator, as it went out of program, this led to it being reset to 20℃ with no light exposure for 5 days, after which we noticed it had changed. Which led to a stunted development compared to the planned schedule, due to this the experiment had to be postponed by a week. Another is the amount of casualties that occurred, both during the eggwaves and the choice arena experiment as there were, when needed, only 12 adult females present on the eggwave for the choice arenas, instead of the aimed for 150. After this a mixed population was used to start the experiment, however only 60 unsynchronised mites were available. As mentioned the deaths during the experiment were also high, of the 72 used mites, 42 died, this was partially caused by the potato leaves desiccating too fast, even with the taken precautions, which means only 30 were still able to be used for the main focus of the report.
The performance experiment was planned to run longer to also properly assess mortality, however due to the time constraints we were only able to run it for a total of 12 days, of which we checked 10.

To continue the research we would suggest taking more time, in which mistakes can be corrected. As well as not pooling all the data from the fourth day together like we did, we only did this to counteract the extremely low number of mites remaining. It would give better results when not pooled as it can give an indication about if it is just because of the host plant or learned behaviour, in our case the lemon was chosen very few for oviposition, thus it could mean the plant itself also plays a role in this.
Another point that could be taken up with this topic is the fact that mites grown on a certain substrate might develop adaptations to completely different plant hosts.

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Appendix

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

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

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

Eggs Hatched													
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**		**Substrate**	**Total Oviposition**
Bean (Control)	0	0	1	13	33	35	38	41	41	41		**Bean (Control)**	42
Lemon	0	0	0	1	1	1	1	1	1	1		**Lemon**	14
Potato	0	0	5	8	14	14	14	15	16	17		**Potato**	28
Rose	0	0	0	3	11	17	17	18	18	20		**Rose**	26