

Investigating aphid resistance in
Lactuca sativa
to *Myzus persicae*.

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Research question: Investigating aphid resistance in *Lactuca sativa* to *Myzus persicae*.

Abstract

Myzus persicae (peach potato aphid) is a major pest species in the UK. It transmits plant viruses to crops, which is synonymous to mosquitoes transmitting malaria to humans. They feed on a variety of hosts, which makes them a substantial threat to agriculture and it is the reason why they were selected for this Investigation (Rothamstead Research, 2014).

The aim of the experiment was to investigate what the defence mechanisms of the resistant lettuce cultivar (Lj10224 from Dr Paul Hand at Harper Adams University), if there were any, in comparison to the susceptible lettuce cultivar (Soleison from Ed Moorhouse at G's Global®). I hypothesised a difference in aphid behaviour and a presence of defence mechanisms in the resistant lettuce in comparison to the susceptible lettuce. This is important as the experiment may begin to identify whether this resistant lettuce's defence mechanism can be applied to other crop species.

The Electrical Penetration Graph technique provided data on the length of time the aphids spend phloem feeding, in pathway and not penetrating. Therefore this experiment's scope only covers the passive plant defence mechanisms to aphids and does not extend to plant communication or attracting predators of aphids. A behavioural study of the aphids was also conducted, showing the aphids' satisfaction with the lettuce cultivar and their antennae and locomotive behaviour.

The results showed that there was no presence of short-term defence mechanism. The EPG eliminated the presence of passive defence mechanisms such as a thick cuticle or the phloem being difficult to locate. There was no significant difference in the behaviour of the aphids in the behavioural study. This shows that the aphids were not deterred from the resistant lettuce in comparison to the susceptible lettuce. This led me to reject my hypothesis for the limited scope of this experiment.

Rothamstead Research (2014) 'Aphid species', Insect Survey Resources, Available <http://www.rothamsted.ac.uk/insect-survey-resources/aphid-species> [accessed: 8/09/2014]

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I would like to thank Dr Laura Vickers for supervising me during this experiment and giving me access to the School of Biosciences lab in the University of Birmingham. I am grateful to the University of Birmingham for providing me with the STEM placement where this research was conducted. I would also like to thank Trina and Stephie for working with me during the STEM placement and sharing the workload of the experiments and data collection

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Glossary of terms

(Vickers, 2011)

Alate: Winged aphid morph.

Apterous: Wingless aphid morph.

EPG: Electrical penetration graph.

Stylet: Thin tubular protrusion used to pierce plant tissue and feed.

Viviparous: Embryo grows inside the female body and gains nutrition from the body. Female gives birth to live young.

Cultivar: A plant variety that has been produced through selective breeding.

1 Introduction

1.1 Plant defence mechanisms

1.1.1 Passive

Passive plant defence is based on more permanent adaptations to the plant, which protect it against pests. For example a plant might not grow in an area where there is a greater abundance of pests. Another example is if a plant has a very thick and tough cuticle, which makes it difficult for stylets to penetrate (Vickers 2011). Plants may have toxins in their phloem, which discourages pests from feeding on them.

1.1.2 Active

Active plant defence is based on short-term responses to the environment. For example, when an aphid penetrates into the phloem of a plant, a change in the conformation of proteins may occur and a callose plug forms in the phloem or toxic secondary metabolites are released (Vickers, 2011). There may be hormonal responses, which cause volatiles to be released by the plant to attract the predators of the aphid, for example, parasitic wasps. Plant to plant communication can occur to inform other plants of any threats so they can activate their defence mechanisms.

1.2 Aphids

Myzus persicae has an alate and apterous morph. They usually grow between 1.0mm and 2.1mm. They can be green, yellow, or even red. These aphids are polyphagous and therefore it can feed on a variety of hosts, such as potatoes, sugar beets lettuce, brassicas and legumes. This makes this species quite a big problem for farmers since is a vector for more than 120 viruses, which includes the potato leaf roll virus, beet western yellow virus and lettuce mosaic virus. *M.persicae* is a phloem feeder. It penetrated into plants phloem with its stylet. Since the fluid in the phloem is under pressure it enters the aphid's food canal. The aphid mainly utilizes the amino acids in the phloem sap.

Vickers, L.H. (2011) 'Aphid responses to drought: A combined physiological and transcriptomic approach', Birmingham university research archive, pp. 16-17

Ibid, pp.100

Ibid, pp.38

Myzus persicae reproduces asexually during the summer, when all the aphids are female. They are also viviparous. The aphids used in this experiment are kept in 18 hours of sunlight and 6 hours of dark, in order to keep them female since sexual forms are triggered by light and dark cycles. Sexual morphs are dependent on day/night cycles according to Dr Laura Vickers, School of Biosciences, University of Birmingham. There is a telescoping of generations within the *M.persicae* as the mother contains both the daughter and the granddaughter growing within them, in order to influence their growth to adapt rapidly to the environment. During the winter, the mother asexually reproduces to produce a daughter and a son. These aphids mate to produce an egg (zygote), which can survive through the low temperatures in winter (Rothamstead Research, 2014).

M. persicae are not cold tolerant, so usually their population is kept under control since many die out over the winter. However, earlier this year, the temperatures in winter were not as low as normal and more aphids have survived. This caused the population to grow very large this summer (Rothamstead Research, 1968)

1.3 Aims

By using the electrical penetration graph, we hope to identify what the resistant lettuce cultivar's defensive mechanism is. We analyse the fluctuations in the voltage traces of the EPG and compare them to that of a susceptible lettuce cultivar. By doing this we can find out the feeding behaviours of the aphids on the resistant lettuce in comparison to susceptible lettuce.

2 Methodology

2.1 Hypothesis

I hypothesise that there are plant defence mechanisms against aphids in the resistant cultivar in comparison to the susceptible cultivar.

2.2 Predications

The aphids will spend longer inside the plant tissue of the susceptible lettuce cultivar than the resistant lettuce cultivar. This may be due to defence mechanisms on the surface of the leaves of the resistant lettuce cultivar, or defences in the plant tissue itself, which makes the aphid retract its stylet or prevents the aphids from finding the phloem. Consequently, I believe that the aphid will spend less time not penetrating while on the susceptible lettuce than the resistant lettuce.

2.3 Independent variable

The independent variable is whether the aphids feed on susceptible or resistant *Lactuca sativa* cultivar while the EPG is running. 12 plants of each kind will be used and one aphid will be used per plant (24 aphids in total).

Rothamstead Research (2014) 'Aphid species', Insect Survey Resources, Available: <http://www.rothamsted.ac.uk/insect-survey-resources/aphid-species> [accessed: 8/09/2014]

2.4 Dependent Variables

Variable	Why is this important?	How will it be measured?
Time aphid spends phloem feeding/ s	If significantly less time is spent phloem feeding by the aphids between the susceptible and resistant lettuce would suggest that there is an effective defence to aphids in the resistant cultivar.	The waveform that suggests ingestion and phloem feeding is E2 (refer to waveform diagram on page 11). Therefore this variable will be measured by how much time on the trace is E2 .
Time spent in pathway/ s	If the aphids spend more time in pathway (proportional to time spent not penetrating or phloem feeding) in the resistant cultivar than the susceptible, it would suggest that this is where the resistant cultivar's defences are.	The waveform that suggests the aphid's stylet is in pathway is C (refer to waveform diagram on page 11). Therefore this variable will be measured by how much time on the trace is C .
Time spent not penetrating/ s	If the aphid spends more time not penetrating (proportional to time spent in pathway or phloem) on the resistant lettuce than the susceptible lettuce, it may suggest that the aphid defence of the resistant lettuce is located on the surface of the leaf, preventing the aphid from penetrating.	The waveform that suggests not penetrating is np (refer to waveform diagram on page 11). Therefore this variable will be measured by how much time on the trace is np .

2.5 Controlled variables

Controlled variable	How will this be controlled or measured?	Justification of measurement.
Organism: Type of lettuce.	Both groups of lettuce (susceptible and resistant) are iceberg lettuces. The susceptible lettuce cultivar is called Soleison and it is from Ed Moorhouse at G's Global®. The resistant cultivar is called Lj10224 and it is from Dr Paul Hand at Harper Adams University.	Similar varieties of lettuce were used so that the plants would be at the same stage of growth at the time of the experiment. We assumed that if the lettuce cultivars were both iceberg, then they would have similar growth rates.
Growth: If there was a difference in the stages of growth of the two lettuce cultivars being used.	The number of true leaves per plant were counted (excluding cotyledons). A t-test will be conducted to see if there was a significant difference between the stages of growth of the two lettuce cultivars.	The width or height of leaves was not used because the leaf shapes were different between the two cultivars. Counting the number of true leaves is an accurate representation of the stage of growth the lettuce cultivars are at.
Organism: Aphid species	O clones of <i>Myzus persicae</i> were used. Therefore they are genetically identical females. Only mature females were used (the largest aphids). All the aphids were from the Rothamsted Institute and were feeding on <i>Brassica rapa chinensis</i> (pak choi). Furthermore, only the apterous (wingless) morph of the aphid was used.	Different clones of the same species have been known to respond differently to environmental change. Therefore we have used the same clone to avoid observing different behaviours associated to the clone of the aphid.
Growing condition of the plants, such as light intensity, humidity, temperature, and volume of water used when watering the plants.	For the EPG experiment, the resistant lettuce cultivar was planted on 03/07/2014 and the susceptible lettuce cultivar was planted on 18/07/2014. The EPGs were done 3 weeks after the cultivar was planted (24/07/2014 and 08/08/2014 respectively). For the aphid behaviour experiment, both cultivars of lettuce were planted on 25/07/2014 and grown for 2 weeks in a plant growth room. The plant growth room maintained a constant temperature and humidity. Both cultivars were kept in the same tray, and therefore each plant pot received an equal volume of water and they were exposed to the same intensity of light.	Growing conditions affect the rate of growth of plants. Light intensity is a rate-limiting factor for photosynthesis, which makes glucose (monomer of cellulose and is respired to provide energy for growth). Temperature will affect plant growth. As the temperature increases, the rate of enzymatic reactions increases until the optimum temperature is reached, then the enzymes denature at any higher temperatures. An increase in the rate of metabolic reactions may lead to an increase in the rate of plant growth.

2.6 Making EPG Pins

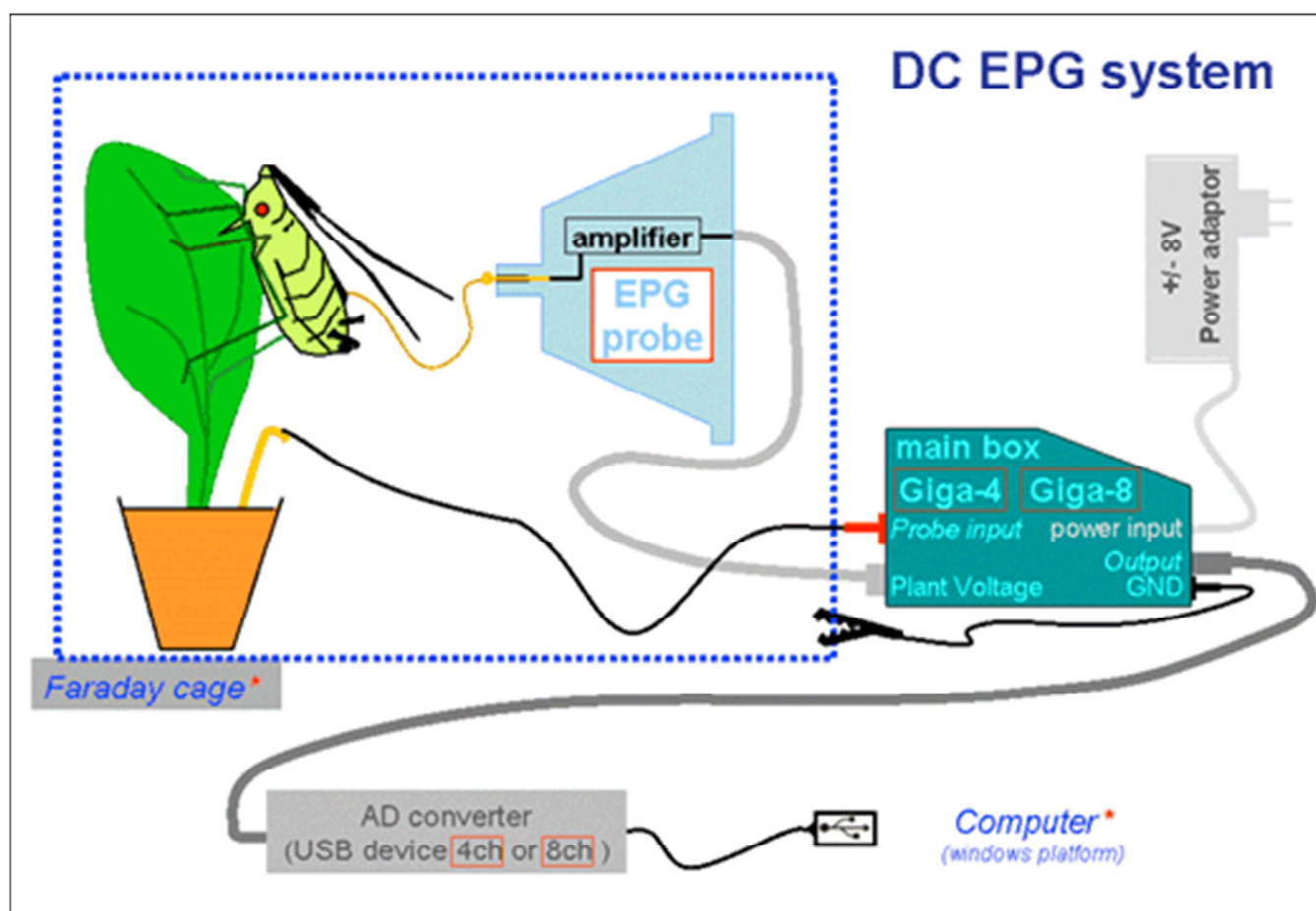
1. Use pins that fit the EPG machine
2. Wrap copper wire around the end of the pin
3. Apply silver paint (safety protocol in appendix pg. 32) to the coil
4. Silver paint can be thinned with Butyl Acetate (safety protocol in appendix pg. 32). The consistency should be thin enough to easily use and apply, and thick enough to maintain adhesiveness
5. Make a small loop at the end of the copper wire
6. Feed a 100mm length of 0.25mm gold wire through the loop and twist the wire around itself (sharp instruments hazard).
7. Apply silver paint to this joint
8. Wrap the gold wire around the silver paint joint
9. Apply more silver paint
10. Allow to dry
11. Make a curve at the end of the gold wire
12. Apply paint to get a blob on the end of the gold wire. This is where the aphids are stuck to

2.7 Plant growth conditions

1. Do not enter plant growth room after being in any insect room
2. Never introduce plants into the growth room (the only plants that should be in the growth room are the ones sown there)
3. Check plants regularly for pests or signs of infection
4. Remove unwanted plants (excess foliage attracts pests)
5. Fill 12 plant pots with fertile soil
6. Place them on a tray
7. Fill tray with 1 litre of water
8. Pour 100ml into each plant pot
9. Place lettuce cultivar on top of the soil and push down 10mm (make sure seed is covered with soil)
10. Label plant pots with date planted and species name

2.8 EPG Protocols

1. Dip blob of silver paint on the end of the gold wire into more silver paint
2. Quickly stick this blob onto a fat aphid by gently lowering the blob on its back
3. Wait 15 seconds to allow the paint to dry and stick to the aphid and to allow the aphid to retract its stylet so it isn't damaged while pulling the aphid off the leaf.
4. Attach pin onto EPG
5. Make sure the plant is watered well before EPG
6. Put a pin in the soil of the plant pot being tested.
7. Plug the pin with the gold wire into the EPG
8. Gently place the aphid onto a leaf
9. Adjust the gain and voltage of the channel on the amplifier so that the dial fluctuates about 0 and between +5V and -5V.
10. Repeat steps 1-9 for all the plant pots
11. Start the application 'stylet+d'
12. Select a location to save the trace
13. Set the duration of time that the trace will run
14. Click the start button
15. Monitor every 15 minutes for the first hour to make sure the aphids have not fallen off the leaves.



The EPG technique provides us with quantitative data. The technique makes the aphid, plant and recording software a circuit, by having a lead inside the soil of the plant pot and the other end of the circuit attached to the aphid. If the aphid penetrates into the plant tissue, the circuit will close and therefore a waveform will be recorded on the recording software. The waveform depends on which tissue of the plant the aphid's stylet is in. For example, if the stylet penetrates a cell membrane, the membrane potential changes, which leads to the recorded voltage falling. The waveforms produced by the EPG are amplified using a Giga- 8 amplifier before they are recorded on the computer (Vickers, 2011; Tjallingii, 2014) .

From the traces collected by the recording software 'stylet+d' and it is analysed by the software 'stylet+a' on Windows OS. Both the recording and analysis software are available on <http://www.epgsystems.eu/> [8/09/2014] and developed by Freddy Tjallingii, (Wageningen Agricultural University, The Netherlands). The analysed traces are transferred to a spreadsheet where the cumulative time for each behavior such as phloem feeding or not penetrating is calculated.

Vickers, L.H. (2011) 'Aphid responses to drought: A combined physiological and transcriptomic approach', Birmingham university research archive, pp. 105-107

Tjallingii, F. (2014) 'Manual Stylet+ v01.2x' , 'Manual-Giga48' , EPG systems, Available: <http://www.epgsystems.eu/downloads.php> [8/09/2014]

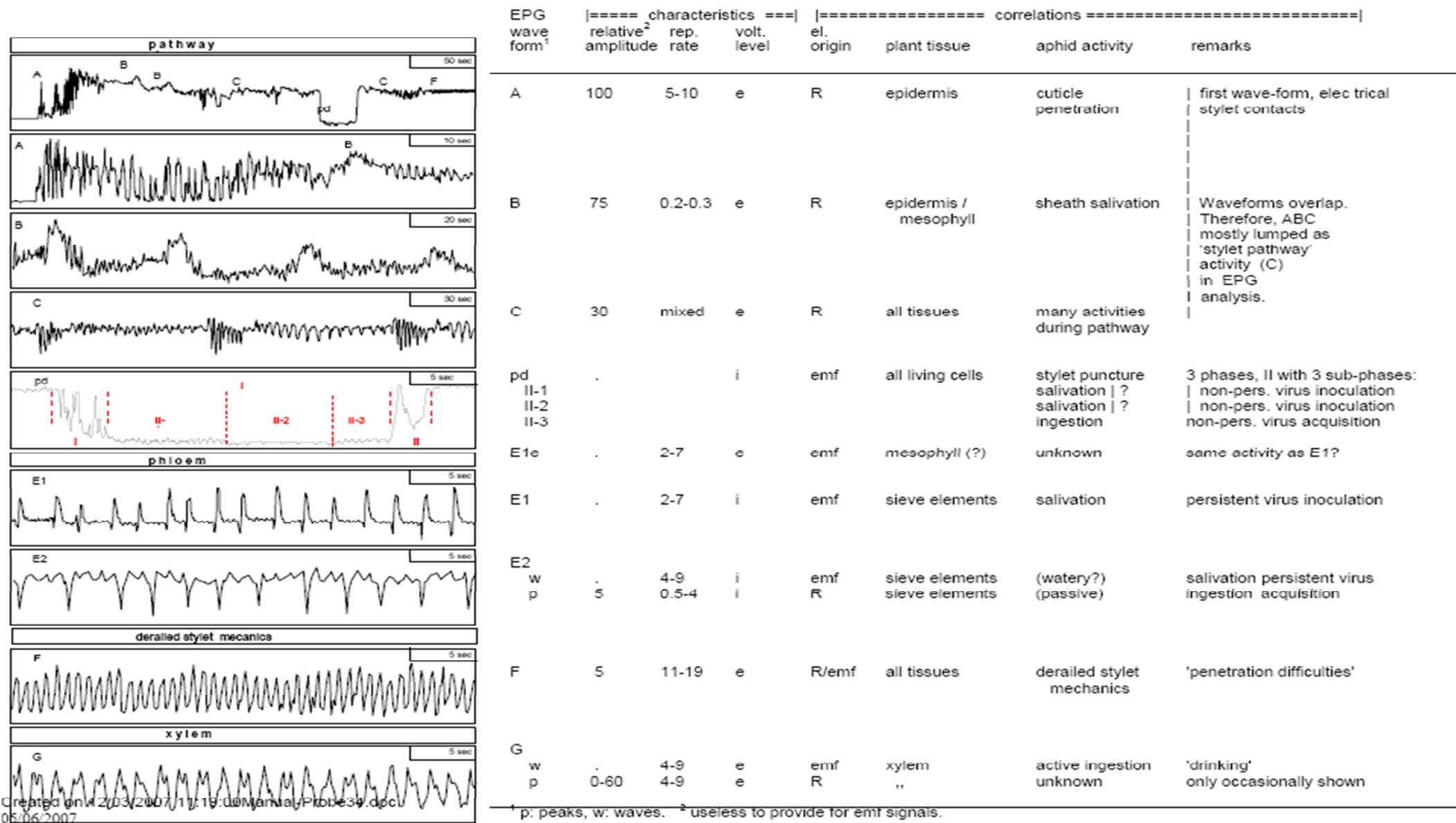


Diagram showing the relationship between the waveforms produced by the EPG and aphid behavior. This can be found on 'Probe 3.4 Manual: Software manual for EPG acquisition and analysis in Windows', by Tjallingii, W. F. (2007) (Vickers, 2011)

Vickers, L.H. (2011) 'Aphid responses to drought: A combined physiological and transcriptomic approach', Birmingham university research archive, pp. 108

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3 Data

(Raw data in appendix)

3.1 Plant growth data

Number of true leaves- 2 nd week		
	Susceptible lettuce (1/8/14)	Resistant lettuce (18/7/14)
	2	2
	1	2
	1	2
	1	3
	1	2
	2	2
	2	2
	2	3
	2	2
	2	2
	2	2
	2	2
Mean= $\frac{\sum x_i}{n}$	1.67	2.17
Standard deviation= $\sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$	0.49	0.39
Number of true leaves- 4 th week		
	Susceptible lettuce (1/8/14)	Resistant lettuce (18/7/14)
	4	3
	4	4
	3	3
	3	3
	3	4
	3	3
	3	4
	4	4
	4	4
	3	4
	3	4
	3	5
Mean= $\frac{\sum x_i}{n}$	3.33	3.75
Standard deviation= $\sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$	0.49	0.62

A t-test will be used to process this data since we need to calculate if there is a significant difference between the means of the number of true leaves on the resistant and susceptible lettuce. If there is a significant difference, the two cultivars are not on the same stage of growth and therefore the variable is not controlled and the results will be inaccurate and not reliable.

Hypothesis: There will be no significant difference between the mean number of true leaves on the susceptible and resistant lettuce cultivar after 2 and 4 weeks of growth.

Null hypothesis: There will be a significant difference between the mean number of true leaves on the susceptible and resistant lettuce cultivar after 2 and 4 weeks of growth.

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

2 weeks

$$t = \frac{|1.67 - 2.17|}{\sqrt{\frac{0.49^2}{12} + \frac{0.39^2}{12}}}$$

$$t = 2.76$$

4 weeks

$$t = \frac{|3.33 - 3.75|}{\sqrt{\frac{0.49^2}{12} + \frac{0.62^2}{12}}}$$

$$t = 1.84$$

- Degrees of freedom = $(n_1 + n_2) - 2$
- Degrees of freedom = 22

The calculated t-value is 2.76 for the cultivars at 2 weeks. The critical value for $p=0.05$ (5% probability that the difference occurred by chance) at 22 degrees of freedom is 2.07 (Damon et al, 2007). Therefore the calculated t value is more than the critical value. This means we must reject the hypothesis and accept the null hypothesis that there is a significant difference between the mean number of true leaves on the resistant and susceptible lettuce cultivars after two weeks of growth.

The calculated t-value is 1.84 for the cultivars at 4 weeks. The critical value for $p=0.05$ (5% probability that the difference occurred by chance) at 22 degrees of freedom is 2.07 (Damon et al, 2007). Therefore the calculated t value is less than the critical value. This means we must reject the null hypothesis and accept the hypothesis that there is no significant difference between the mean number of true leaves on the resistant and susceptible lettuce cultivars after four weeks of growth.

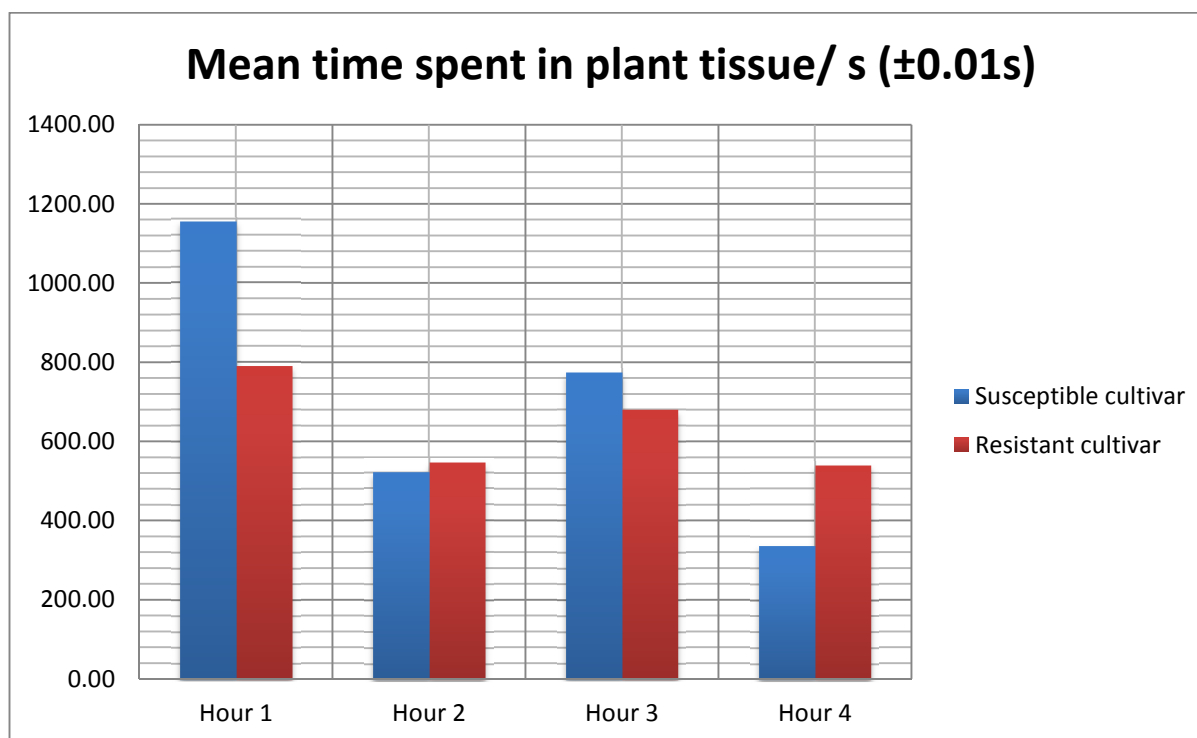
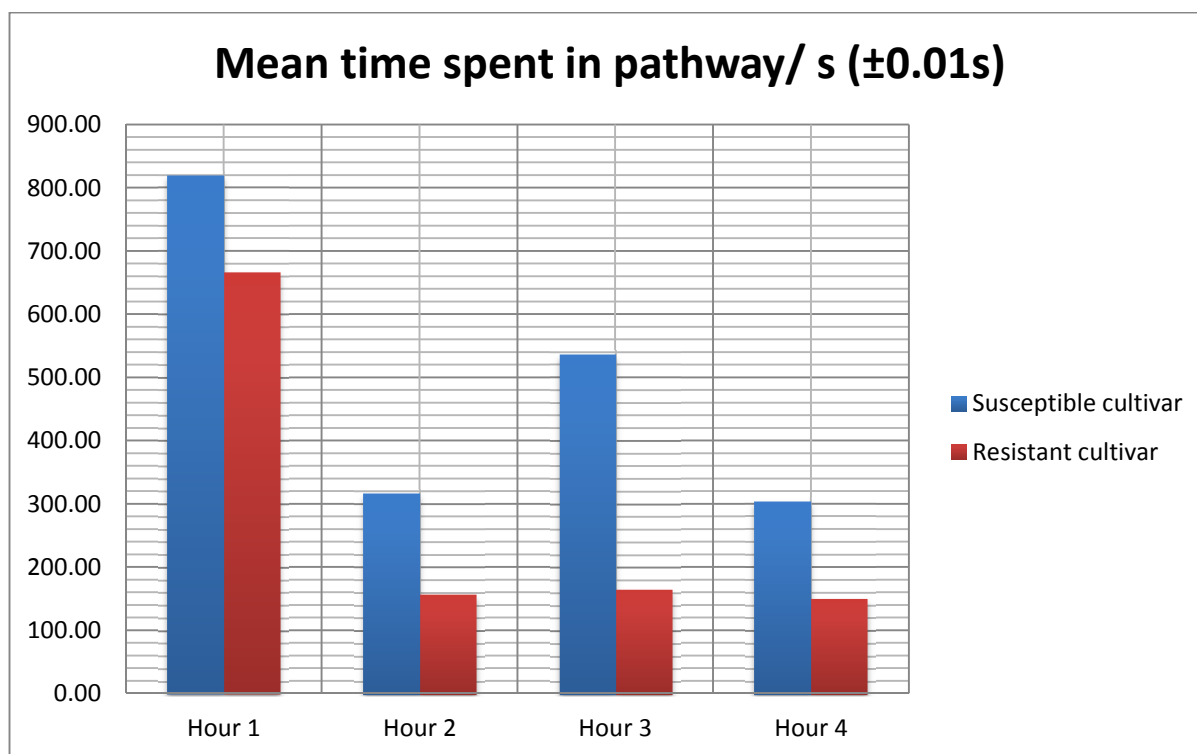
This suggests that the variable of the stage of growth of the plants were controlled when the EPG was conducted, making the results more reliable. However this also suggests that the resistant lettuce cultivar grows at a faster rate during the first 2 weeks of growth than the susceptible cultivar.

Damon, A. McGonegal, R. et al (2007) 'Pearson Baccalaureate Biology, Essex: Pearson Education Limited, pp. 7

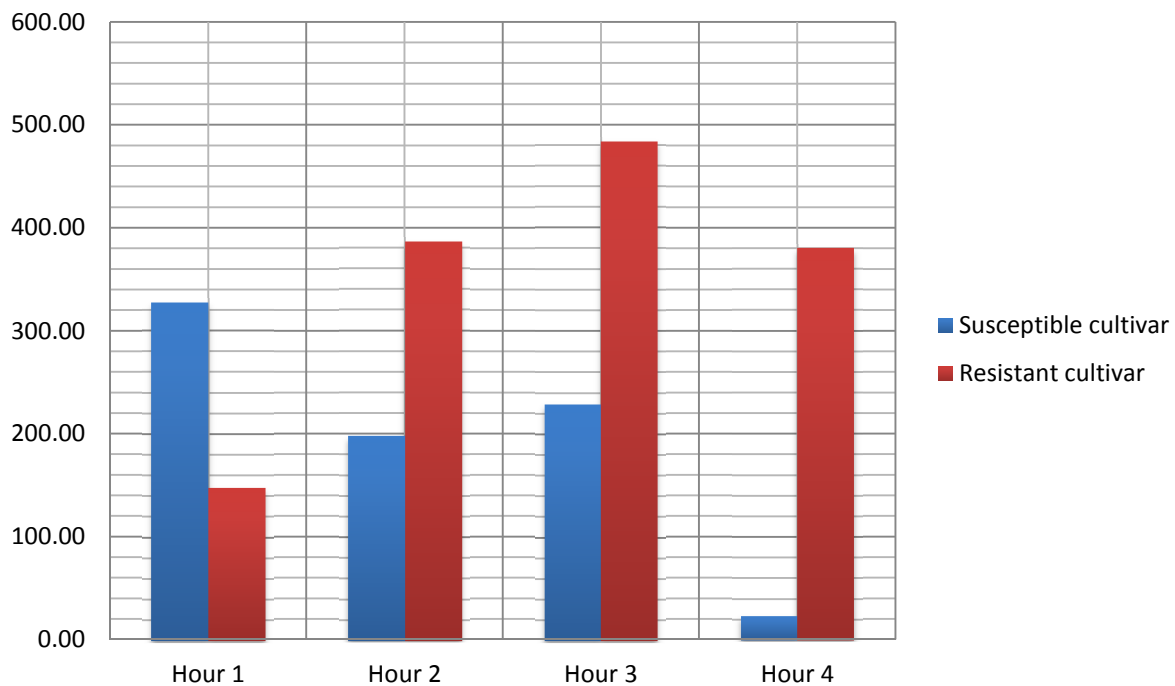
Ibid

4 Data presentation

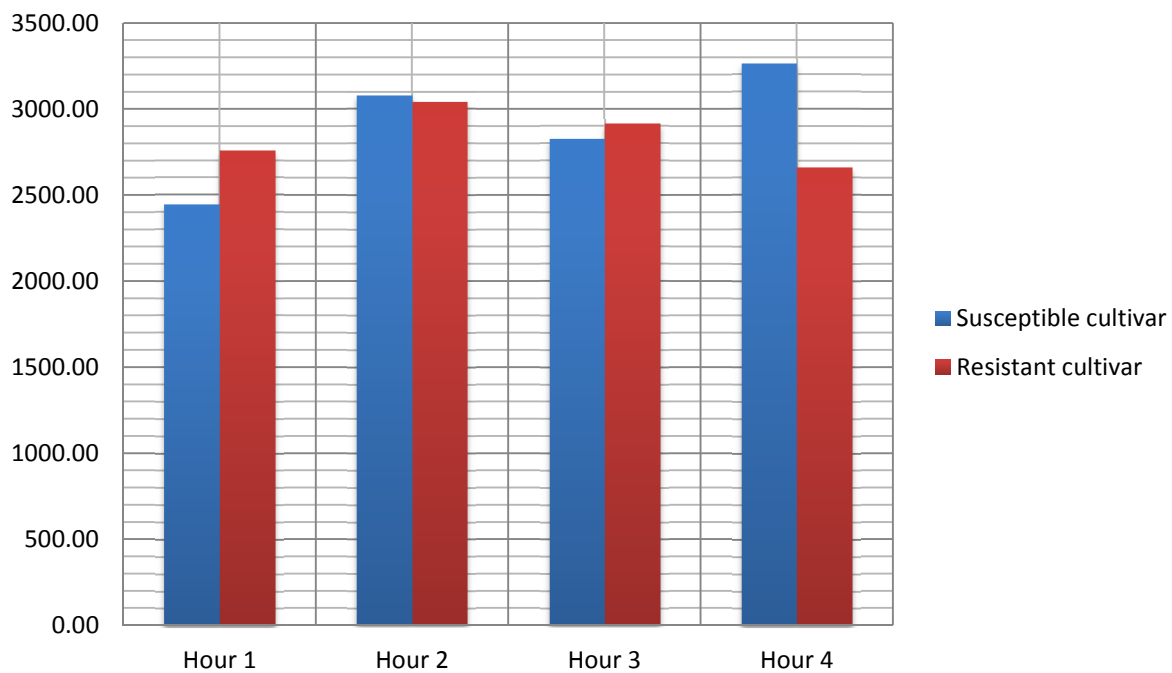
4.1 Bar charts comparing mean time spent doing an activity by the aphids on the susceptible and resistant cultivar.



Mean time spent phloem feeding/ s ($\pm 0.01s$)



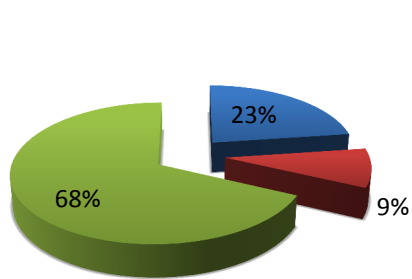
Mean time spent not penetrating/ s ($\pm 0.01s$)



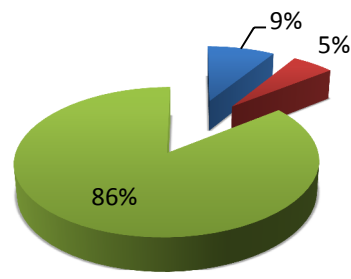
4.2 Pie charts showing the relative length of time spent by the aphids doing each activity

Susceptible cultivar

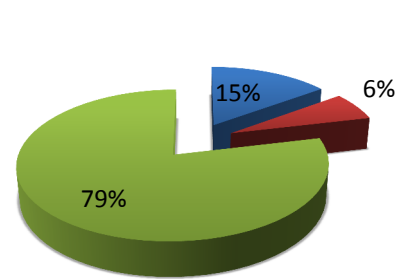
Hour 1



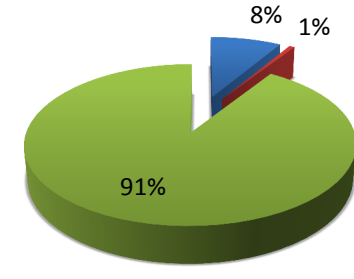
Hour 2



Hour 3



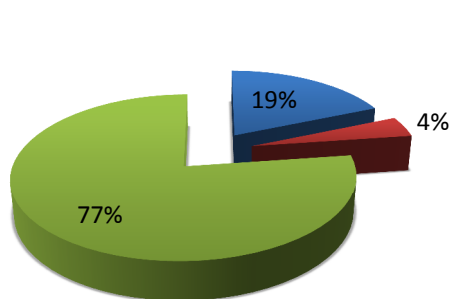
Hour 4



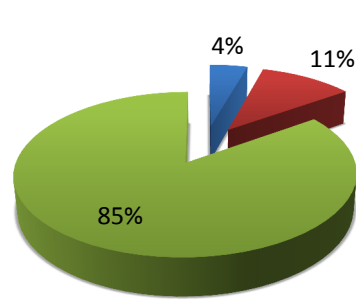
- Time spent in pathway
- Time spent Phloem feeding
- Time spent not penetrating

Resistant cultivar

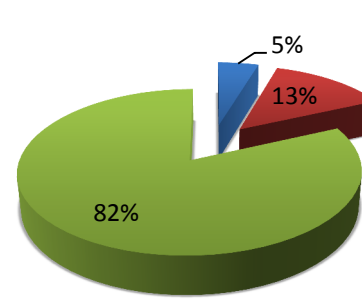
Hour 1



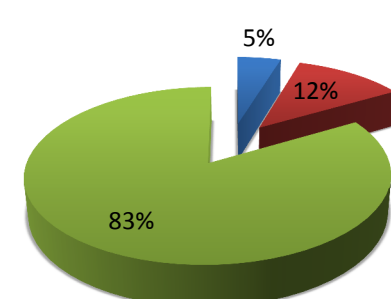
Hour 2



Hour 3



Hour 4



5 Data analysis

The aphids spend more time in pathway on the susceptible lettuce cultivar than the resistant lettuce cultivar. This difference is greatest in the third hour, however it is largely consistent. The mean time spent in pathway for the aphids on both the resistant and susceptible cultivar is greatest in the first hour, occupying almost a quarter and a fifth of the first hour on the susceptible and resistant lettuce respectively. It then decreases to 160 seconds and remains there consistently for the second, third and fourth hour on the resistant lettuce, whereas the time spent in pathway on the susceptible lettuce is greater.

The mean time spent in the plant tissue is greatest in the first hour on both cultivars. However there seems to be no consistent difference between the total time spent in plant tissue between the lettuce cultivars.

Interestingly, the mean time spent phloem feeding is greater on the resistant lettuce cultivar than the susceptible lettuce, except in the first hour. For the last three hours, on average, 12% of time is spent phloem feeding on the resistant lettuce, which is three times greater than the average for the last three hours of the EPG on the susceptible lettuce.

There seems to be very little difference in the mean time spent not penetrating between the lettuce cultivars. For both cultivars, it is the least in the first hour.

6 Conclusion

From this data we can conclude that there seems to be no presence of a short-term plant defence mechanism against the aphids on the resistant lettuce cultivar in comparison to the susceptible lettuce cultivar. In fact the results shown on the pie charts suggest that the phloem may be easier to find in the resistant lettuce in comparison to the susceptible lettuce. This can be deduced by the fact that the aphids spend relatively less time in pathway (searching/ probing for the phloem) and more time phloem feeding on the resistant lettuce than the susceptible lettuce. This result can be compared directly between the two lettuce cultivars since there is very little difference in the time spent in plant tissue between the two cultivars.

Since prior work has been done at Adam's University to show that the resistant cultivar is actually resistant, these results may suggest that there is the resistant lettuce has a long-term effect on the aphids, for example it might reduce their rate of reproduction. Another explanation is that the organic material in the phloem of the resistant lettuce cannot be fully digested by the aphids, therefore starving them. However this would have to be tested by comparing the chemicals in the phloem of a resistant and a susceptible cultivar.

From the results, we can rule out there being a passive defence mechanism on the surface of the resistant lettuce since there was very little difference between the length of time spent in plant tissue in the susceptible and resistant lettuce. Therefore there seems to be nothing hindering the aphids from penetrating the surface of the resistant lettuce leaf.

We can rule out that there are chemicals, such as toxins, in the phloem of the resistant plant (passive defence) that repelled the aphids since, on average, aphids on the resistant lettuce spend more time phloem feeding than on the susceptible lettuce.

We cannot rule out active defence mechanisms such as plant communication using volatiles because the scope of this experiment does not extend this far due to the limited number of plants used.

7 Evaluation

Limitation	Predication (effect on results)	Improvement
Circuit was shorted due to gold wire touching the end of the plug of the EPG.	This lead to there being no trace on the EPG for that plant pot. This meant that a fewer number of traces could be used to gain data, therefore reducing the precision of the data.	Check the gold wire is free from the pin to avoid shorting the circuit. This should be checked every hour since the wire moves with the aphid.
Aphids were damaged while sticking them to the gold wire with silver paint and while removing the aphids from the pak choi.	This meant the aphids, which were damaged, could not move properly or penetrate the surface of the leaves with it's stylet if it was damaged. This may explain why such a large proportion of the time was spent not penetrating. This limitation would produce random errors since some aphids might be damaged and others not.	The aphids should be stuck to the gold wire with silver paint while they are still, on the pak choi. Before pulling the aphid off, you should wait until the aphid moves, to make sure its stylet is not in the plant tissue. Another method would be to agitate the aphid with a wet paintbrush and transfer them to a petri dish while they are moving, and then stick the gold wire to them. This would avoid the aphid's stylet still being in the plant tissue when pulling them off.
Aphids were fed on pak choi constantly until the experiment.	This meant that the aphids might have been full at the time of the experiment and not willing to feed anymore when they were placed on the lettuce leaves. This may have led to the aphids spending less time phloem feeding or in pathway, searching for the phloem, since they might be full. Therefore, this limitation introduced a systematic error to the experiment and reduced the accuracy of the results.	The aphids should be kept off any food source for an hour before the experiment.
The resistant lettuce was only tested against one type of susceptible lettuce	The aphids may not like feeding on the particular type of Soleison from Ed Moorhouse at G's Global®. This would lead to the results showing no significant difference in the aphid's behaviour and feeding between the resistant and the susceptible lettuce cultivars. This would introduce systematic error and reduce the accuracy of our results as well as preventing us from forming definite conclusions about the resistant lettuce.	A variety of susceptible lettuce cultivars should be tested in comparison to the resistant cultivar. This will also improve the reliability of our results by increasing the number of repeats, as well as reducing the likelihood that the aphid does not like to feed on one type of lettuce for some reason.

Aphid behaviour experiment

8 Methodology

8.1 Hypothesis

I hypothesise that the susceptible lettuce cultivar will score higher on the plant satisfaction scoring test than the resistant lettuce cultivar. Furthermore I predict that there will be a higher frequency of aphids with their antennae behind them and still on the susceptible lettuce than the resistant lettuce.

8.2 Independent variable

The independent variable is whether the lettuce cultivar, on which the aphids were kept for the experiment, was susceptible or resistant to aphids. There will be 4 aphids on each plant to avoid overcrowding. Three lettuce plants of each cultivar will be used, therefore 12 aphids will be sampled per lettuce cultivar.

8.3 Dependent variables

Variable	Why is this important?	How will this be measured?
Satisfaction with plant	This will show how satisfied the aphids are on the susceptible and resistant lettuce cultivars. This will give an indication as to whether the resistant cultivar has short-term deterrents against aphids.	Aphid satisfaction with plant scoring.
Aphid behaviour category 1: locomotion	If the aphids are walking around a lot, it means it cannot find a suitable position to probe or penetrate the lettuce tissue with its stylet. If the aphid is still, it has settled and found a satisfactory place to penetrate the plant tissue with its stylet.	Aphid locomotion behaviour scoring (discrete data)
Aphid behaviour category 2: antennae position	If the antennae are behind the aphid, this suggests the aphid is satisfied with its position and it may be phloem feeding.	Aphid antennae behaviour scoring (discrete data)

8.4 Scoring

4 aphids were placed on a lettuce leaf. As soon as they were all on the plant, the timer was started. The aphids were instantaneously sampled every minute for 10 minutes. The following scoring method was used.

8.4.1 Aphid satisfaction with plant

Category	Behaviour	Points
Locomotion	Walking	1
	Still	2
Antennae	Antennae in front of aphid	1
	Antennae above aphid	1
	Antennae behind aphid	3

Every minute, a score for locomotion behaviour and a score for antennae behaviour are taken. At the end of the 10 minutes, the scores are all added up for each aphid and a mean will be calculated for all the aphids on the susceptible lettuce and all the aphids on the resistant lettuce. A t-test will be conducted on these means.

8.4.2 Aphid behaviour

Category	Behaviour	Points
Locomotion	Walking	1
	Still	2
Antennae	Antennae in front of aphid	1
	Antennae above aphid	2
	Antennae behind aphid	3

Every minute, a score for locomotion behaviour and a score for antennae behaviour will be taken. At the end of the experiment the frequencies of each behaviour will be calculated. The proportions of the frequencies of each behaviour will be displayed as discrete data and compared between the two lettuce cultivars.

8.5 Protocol and method

1. Using a fine, wet paintbrush, pick up a mature aphid from its pak choi leaf.
2. Place 4 aphids on the largest leaf of a susceptible lettuce plant.
3. Start the stopwatch and take first score of all 4 aphids for both categories at 0 minutes
4. Repeat every minute for 10 minutes.
5. Repeat steps 1-4 2 more times.
6. Repeat steps 1-5 on a resistant lettuce plant.

9 Data

9.1 Plant growth data

Number of true leaves		
	Susceptible lettuce	Resistant lettuce
	1	1
	2	2
	3	3
	2	2
	2	3
	2	2
Mean= $\frac{\sum x_i}{n}$	2	2.17
Standard deviation= $\sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$	0.58	0.75

A t-test will be used to process this data since we need to calculate if there is a significant difference between the means of the number of true leaves on the resistant and susceptible lettuce. If there is

a significant difference, the two cultivars are not on the same stage of growth and therefore the variable is not controlled and the results will be inaccurate and not reliable.

Hypothesis: There will be no significant difference between the mean number of true leaves on the susceptible and resistant lettuce cultivar.

Null hypothesis: There will be a significant difference between the mean number of true leaves on the susceptible and resistant lettuce cultivar

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$

- $t = \frac{|2 - 2.17|}{\sqrt{\frac{0.58^2}{6} + \frac{0.75^2}{6}}}$
- $t = 0.44$
- Degrees of freedom = $(n_1 + n_2) - 2$
- Degrees of freedom = 10

There is no significant difference between the two means. The calculated t-value is 0.44. The critical value for $p=0.05$ (5% probability that the difference occurred by chance) at 10 degrees of freedom is 2.23 (Damon et al, 2007). Therefore the calculated t value is less than the critical value. This means we must reject the null hypothesis and accept the hypothesis that there is no significant difference between the mean number of true leaves on the resistant and susceptible lettuce cultivars. This means that the stage of growth of the plant is controlled.

9.2 Aphid satisfaction with plant

9.2.1 Table 1: Each aphid's score on satisfaction with susceptible lettuce cultivar

Aphid	Locomotion score	Antennae score	Total score
1	18	15	33
2	16	17	33
3	20	25	45
4	15	17	32
5	18	11	29
6	18	13	31
7	20	18	38
8	18	17	35
9	17	11	28
10	20	13	33
11	15	13	28
12	21	19	40
Mean=	$\frac{\sum x_i}{n}$	Mean score:	33.75

Standard deviation= $\sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$	Standard deviation:	5.10
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9.2.2 Table 2: Each Aphid's score on satisfaction with resistant lettuce cultivar

Aphid	Locomotion	Antennae	Total
1	18	21	39
2	21	21	42
3	21	25	46
4	21	29	50
5	21	21	42
6	19	13	32
7	16	11	27
8	18	11	29
9	17	23	40
10	16	13	29
11	20	11	31
12	17	11	28
Mean score:			36.25
Standard deviation:			7.84

9.3 Data processing

A t-test will be used to process this data since we need to calculate if there is a significant difference between the means of the satisfaction scores of the aphids on the susceptible and resistant lettuce cultivars. A t-test can be used because the results are normally distributed.

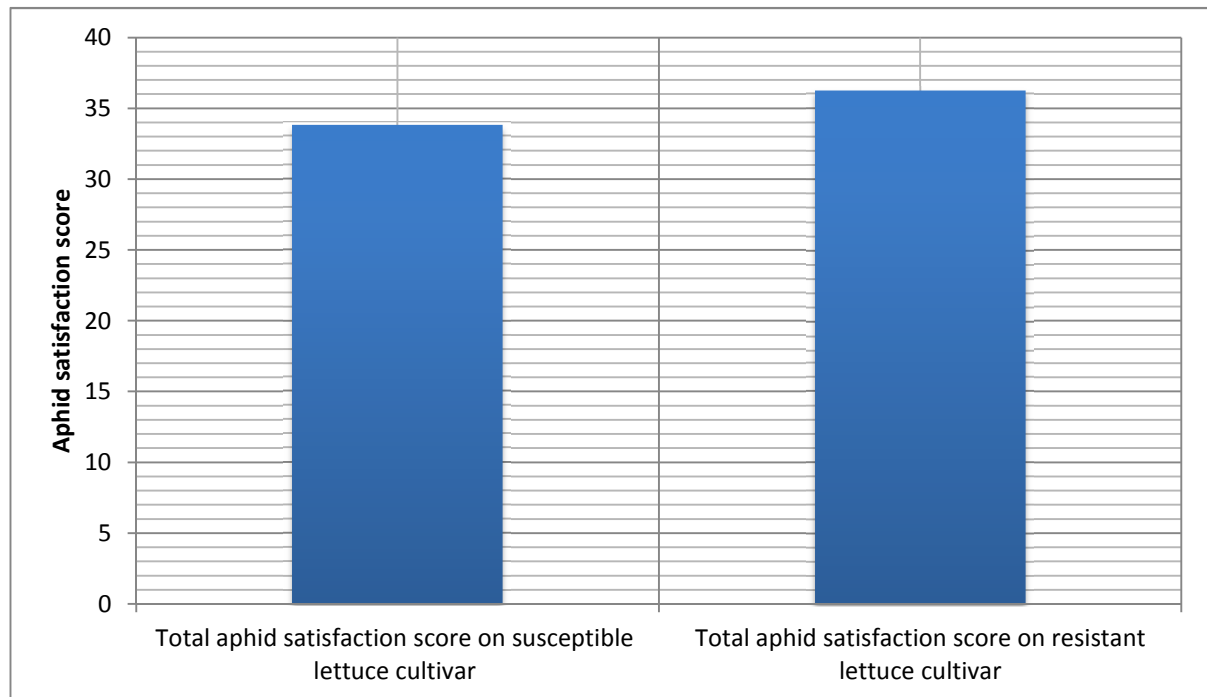
Hypothesis: There will be a significant difference between the mean scores of aphid satisfaction on the susceptible and resistant lettuce cultivar.

Null hypothesis: There will be no significant difference between the mean scores of aphid satisfaction on the susceptible and resistant lettuce cultivar.

- $$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}}}$$
- $$t = \frac{|33.75 - 36.25|}{\sqrt{\frac{5.10^2}{12} + \frac{7.84^2}{12}}}$$
- $t = 0.93$
- Degrees of freedom = $(n_1 + n_2) - 2$
- Degrees of freedom = 22

There is no significant difference between the two means. The calculated t-value is 0.93. The critical value for p=0.05 (5% probability that the difference occurred by chance) at 22 degrees of freedom is Investigating aphid resistance in *Lactuca sativa* to *Myzus persicae*.

2.07 (Damon et al, 2007). Therefore the calculated t value is less than the critical value. This means we must reject the hypothesis and accept the null hypothesis that there is no significant difference between the mean aphid satisfaction scores.



10 Data presentation

10.1 Aphid behaviour

10.2 Raw data

10.2.1 Locomotion

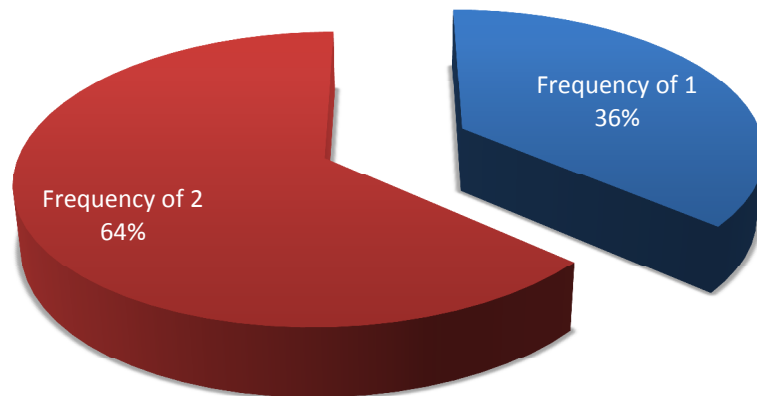
	Frequency of 1	Frequency of 2
Susceptible	48	84
Resistant	39	93

10.2.2 Antennae

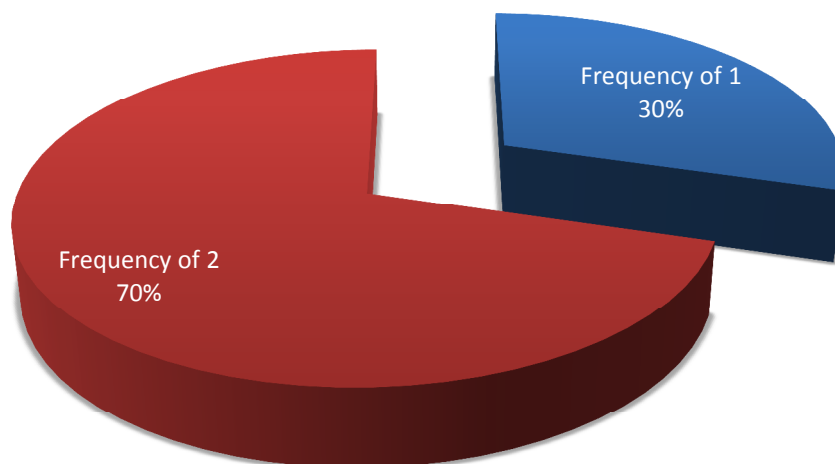
	Frequency of 1	Frequency of 2	Frequency of 3
Susceptible	51	52	29
Resistant	33	60	39

10.3 Locomotion

Locomotion of aphids on susceptible lettuce

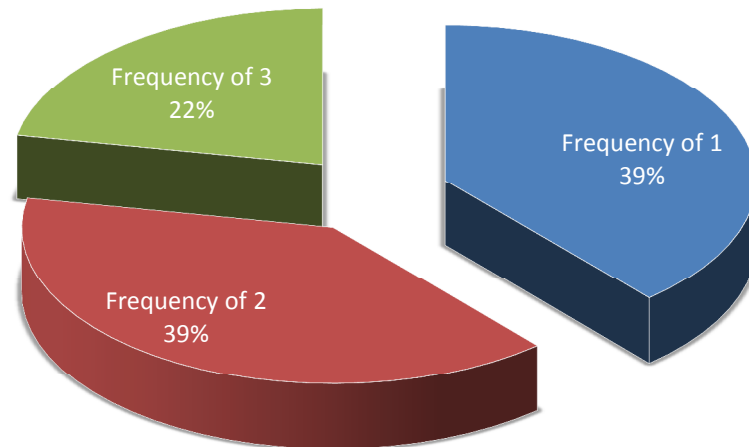


Locomotion of aphids on resistant lettuce

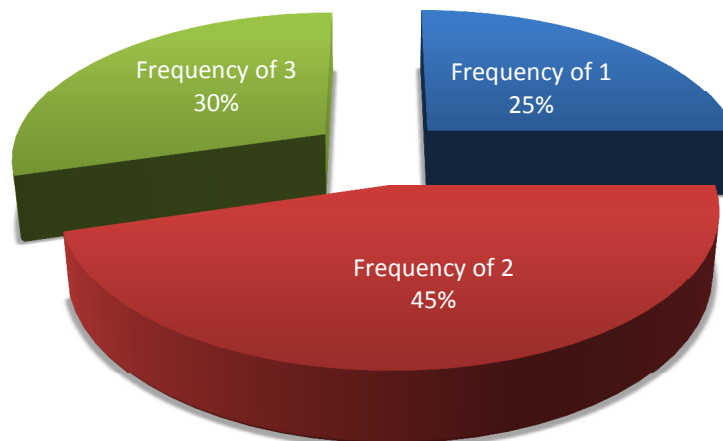


10.4 Antennae behaviour

Antennae behaviour of aphids on susceptible lettuce



Antennae behaviour of aphids on resistant lettuce



11 Conclusion

There was no significant difference between the aphid satisfaction scores of the aphids on the susceptible lettuce and the aphids on the resistant plants. This may suggest that the resistant lettuce reduces the rate of reproduction of the aphids compared to the susceptible cultivar. This suggests the resistant lettuce is not repulsive to the aphids but it may have a long-term effect on the aphid population. This can be seen by the fact that the aphids spend a similar amount of time walking on both the lettuce cultivars. In fact the frequency of walking is 6% greater on the susceptible lettuce than the resistant lettuce. Aphids on the resistant lettuce had 6% greater frequency of being still than the aphids on the susceptible plants and therefore they may be more satisfied with the surface of the lettuce leaf to probe with its stylet.

According to the antennae behaviour, there is a significantly lower frequency of aphids with their antennae's in front of them (14%) on the resistant cultivar than the susceptible cultivar. This antennae behaviour occurs when the aphids are walking. This may suggest that the aphids on the susceptible plants are less satisfied with the surface of the lettuce leaf than the aphids on the resistant lettuce cultivar. This further supports the conclusion that the resistant lettuce cultivar may not have short-term defences against the aphids. The frequency of the antennae being directly above the aphid was 6% higher with aphids on the resistant cultivar than the susceptible. Furthermore, the frequency of the antennae being down, behind the aphid was 6% higher with the aphids on the resistant cultivar than the susceptible. This behaviour can be associated with the aphid phloem feeding. However, this difference in the frequency of the antennae being behind the aphid is negligible. This behaviour was given the highest score on the aphid satisfaction test. Even after that, this 6% difference showed by the antennae behaviour pie chart probably had minimal effect on the satisfaction of the aphid on the plant since there was not significant difference between the aphid satisfaction scores between the two lettuce cultivars.

The fourth limitation was probably the reason behind there being no significant difference between the aphid behaviours on the two lettuce cultivars. The aphids might have been disturbed or agitated when transferring them from the pak choi to the lettuce since it is a change of food source. This limitation probably introduced the largest systematic error in this experiment.

The difficulty in seeing the antennae position and the fact that the aphids kept on falling off the leaves introduced random error into the experiment. Considering these errors and limitation, I think my results are not decisive as to whether there is or there isn't a difference in aphid behaviour on the two cultivars. Therefore, any conclusion made on these results is limited to the scope of this experiment.

In the future, I would have repeated the experiment using 4-week-old lettuce cultivars since they have larger leaves and a magnifying glass to see the aphids' antennae more clearly. This would greatly reduce the random error of the experiment. As with the EPG experiment, I would also take the aphids off the pak choi for a certain amount of time prior to the experiment to ensure they were hungry.

12 Evaluation

Limitation	Predication (effect on results)	Improvement
Difficult to see antennae position	This limitation would introduce random error to the results since mistakes can be made while recording the antennae positions. This would reduce the reliability and the precision of the results.	During the experiment, view the aphids on the lettuce through a magnifying glass to view the antennae position more clearly. This will reduce the random error, which was introduced by errors made by recording the incorrect antennae behaviour.
Assuming a link between aphid satisfaction and their behaviour	We have assumed that there is a link between the locomotion and antennae behaviour and the satisfaction of the aphid with the lettuce. This allowed us to calculate a satisfaction score, however this does not take into account whether the aphid is constantly probing for a phloem to feed on while it is still, or whether it is phloem feeding. Therefore, any conclusions made on this assumption may be less valid.	Aphid satisfaction scores should be based on the length of time an aphid is phloem feeding, or how long it takes for an aphid to find the phloem. This will provide results, which are more valid to make a conclusion about aphid satisfaction. This can be done by using an EPG.
The leaves of the lettuce cultivars were quite small, therefore aphids fell off them a few times during the experiment.	Aphids falling off the lettuce leaves introduced random errors since aphids may have got damaged when they fell off, or lost their position on the leaf, which they were satisfied with. Furthermore this made it hard to measure the aphids every minute since it took a long time to put the aphid back into the lettuce leaf.	The lettuce cultivars were both 2 weeks old. Waiting another 2 weeks to allow the leaves to grow larger and therefore reduce the likelihood of the aphids falling off.
Aphids were fed on pak choi constantly until the experiment.	This meant that the aphids might have been full at the time of the experiment and not willing to feed anymore when they were placed on the lettuce leaves. This may have led to behaviour that suggests the aphids are unsatisfied with a lettuce cultivar, when it might have just been full. Therefore, this limitation introduced a systematic error to the experiment and reduced the accuracy of the results.	The aphids should be kept off any food source for an hour before the experiment.

13 Bibliography

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14 Appendix

14.1 Raw data

14.1.1 EPG Susceptible lettuce cultivar

	Time spent in pathway/ s ($\pm 0.01s$)				Total time spent inside plant tissue/ s ($\pm 0.01s$)				Time spent phloem feeding/s ($\pm 0.01s$)				Time spend not penetrating/s ($\pm 0.01s$)			
Trace	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4
1	819.55	1315.92	690.08	1995.59	819.55	1315.92	787.67	2162.68	0.00	0.00	79.98	86.64	2757.47	2284.08	2812.33	1437.32
2	3448.20	564.64	79.65	438.25	3600.00	564.64	79.65	438.25	128.65	0.00	0.00	0.00	0.00	3035.36	3520.35	3161.75
3	435.79	0.00	136.65	79.98	435.79	0.00	136.65	79.98	0.00	0.00	0.00	0.00	3164.02	3600.00	3463.35	3520.02
4	2.53	17.44	48.24	0.00	4.22	20.38	50.60	0.00	0.00	0.00	0.00	0.00	3595.78	3579.62	3549.40	3600.00
5	140.91	42.12	2134.93	205.72	1558.29	614.54	2134.93	291.27	1391.89	568.79	0.00	85.16	2041.71	2985.46	1465.07	3308.73
6	632.43	93.17	80.23	0.00	646.57	93.17	80.23	0.00	1.95	0.00	0.00	0.00	2953.43	3506.83	3519.77	3600.00
7	508.88	344.64	182.46	4.43	513.87	350.59	568.57	4.43	0.00	0.00	365.54	0.00	3086.13	3249.41	3031.43	3595.57
8	593.87	31.73	1034.23	4.03	2018.00	1037.77	2625.20	4.03	1421.48	968.88	1587.76	0.00	1582.00	2562.23	974.80	3595.97
9	792.56	442.33	440.43	5.31	792.56	688.41	501.20	45.91	0.00	244.10	24.25	40.01	2807.44	2911.59	3098.80	3554.09
Mean	819.41	316.89	536.32	303.70	1154.32	520.60	773.86	336.28	327.11	197.97	228.61	23.53	2443.11	3079.40	2826.14	3263.72

14.1.2 EPG Resistant lettuce cultivar

	Time spent in pathway/ s ($\pm 0.01s$)				Total time spent inside plant tissue/ s ($\pm 0.01s$)				Time spent phloem feeding/s ($\pm 0.01s$)				Time spend not penetrating/s ($\pm 0.01s$)			
Trace	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4	Hour 1	Hour 2	Hour 3	Hour 4
1	949.55	14.23	114.92	465.20	952.32	14.23	114.92	465.20	0.00	0.00	0.00	0.00	2647.68	3585.77	3485.08	3134.80
2	813.34	0.00	0.00	0.00	847.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2741.04	3600.00	3600.00	3600.00
3	2260.99	1094.22	1023.55	709.16	2555.87	1186.59	3133.39	2701.36	145.22	0.00	1955.71	1920.88	1041.57	2413.41	466.61	898.64
4	424.51	38.46	96.30	22.87	478.45	38.46	564.51	22.87	42.43	0.00	460.63	0.00	3121.55	3561.54	3035.49	3577.13
5	492.61	37.07	14.44	131.93	492.61	37.44	14.67	164.93	0.00	0.00	0.00	22.31	3107.39	3562.56	3585.33	3435.07
6	467.81	132.58	49.72	11.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3132.19	3467.42	3550.28	3588.42
7	465.71	89.90	83.83	0.00	791.96	2152.06	640.16	0.00	309.00	2026.19	493.98	0.00	2808.04	1447.94	2959.84	3600.00
8	121.69	6.40	87.27	16.11	994.59	1475.65	1629.04	1497.56	835.39	1446.80	1439.83	1476.51	2605.41	2124.35	1970.96	2102.44
9	0.00	2.03	25.92	0.00	0.00	2.03	25.92	0.00	0.00	0.00	0.00	0.00	3600.00	3597.97	3574.08	0.00
Mean	666.25	157.21	166.22	150.76	790.38	545.16	680.29	539.10	148.00	385.89	483.35	379.97	2756.10	3040.11	2914.19	2659.61

14.2 Safety considerations

Silver paint- <http://docs-europe.electrocomponents.com/webdocs/001d/0900766b8001d974.pdf>

Butyl Acetate- <http://www.sciencelab.com/msds.php?msdsId=9927114>

Sharp instruments- A razor will be used to cut the copper and gold wire to the appropriate length. Care must be taken while doing this to avoid cuts.