Mechanisms of insect resistance in *Lactuca Sativa*

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Abstract
We researched the natural mechanisms of aphid resistance in lettuce plants. We did this to investigate alternatives to pesticides due to the disadvantages they have, such as running into water sources and potentially harming marine life. We knew that aphids did not reproduce as well on resistant lettuce plants and wanted to find out why, by finding out the possible defence mechanisms within the lettuce (whether they were constitutionally expressed or induced).We conducted this by comparing resistant and susceptible iceberg lettuce cultivars using an observation of aphid behaviour and by using an Electrical Penetration Graph (EPG).We also investigated the effect of age on resistance by comparing three weeks old and six weeks old resistant lettuce plants using EPG. From this, we found out that the natural mechanisms of resistance are likely to be constitutionally expressed. Also, we found that age negatively affects resistance. This is useful for farmers because they can grow resistant varieties of lettuce, and not have to use as much pesticide, making the process more economical. Furthermore, fewer pesticides could be used on younger resistant lettuce plant as they seem to have stronger natural defences.

Introduction

Lactuca Sativa (lettuce) is a common leafy vegetable, with over 23 million tonnes being produced globally in 2010[1]. It is mass produced to be used as a food source, but often has to be treated with pesticides to repel insects such as aphids. There are many disadvantages to using pesticides, for example; the chemicals may run off into water sources, potentially harming marine life, and disrupting food chains. A major issue is the residues that may be present when the plants are eaten by humans. Also, repeated use of pesticides may allow the insects to become resistant to them. A further disadvantage is the cost of producing and distributing the pesticides[2]. Therefore, we have investigated natural mechanisms of insect resistance in lettuces.

There are many possible defence mechanisms in plants. Defences can either be constitutionally expressed (always present), or induced. Examples of constitutionally expressed defences in plants include; hairs of leaves – restricts movement of aphids, thick waxy cuticle – restricts stylet penetration, an abundance of mesophyll tissue – so aphid stylets are not long enough to reach the phloem, surface of the plant is the wrong texture or taste for the aphid to feed, volatiles are given off which repel the aphids, or the plant contains toxins which can confuse or kill the aphid.

Induced defences include; the production of toxins, a callose plug – a blockage of the phloem caused by a flux of calcium ions into the phloem, so the aphids are unable to feed from it.

In resistant varieties of Iceberg lettuce it has been found that aphids reproduce less. This may be due to either constitutionally expressed, or induced defences.

The species of aphid we studied are *Myzus Persicae* (Peach-potato aphids). These are polyphagous agricultural pests, and can transmit viruses to plants. This is why we are looking for the mechanism of resistance in resistant lettuce, so that it can be bred into other varieties of crops. *Myzus Persicae* can reproduce both asexually and sexually. The sexually reproducing aphids produce eggs, usually in winter as the young are susceptible to the cold

weather. If the egg gets bumped (due to overcrowding on a leaf), a change in hormones stimulates the growth of wings, and this forms an allet (winged version of aphids). The aphids we studied reproduce asexually, producing live young, which are genetic clones of the mother. Within each female aphid are a further two generations of aphid – this is known as telescoping of generations. This allows signals containing information about the environment to be passed on to the third generation.

Myzus Persicae feed by penetrating the phloem of the plant, by using their stylets. The stylets need to be able to penetrate the cuticle of the plant, long enough to penetrate through the mesophyll tissue of the plant to reach the phloem, and be able to locate the phloem. It is believed that the aphids locate the phloem by following a carbon/sugar gradient, or signals from the tissues.[3]

The phloem is a transportation vessel in plants, which transports amino acids and sucrose to the tissues. The aphids penetrate the phloem in order to obtain the amino acids.

Aims and Objectives

The overall aim of our project was to determine the natural mechanism of resistance to *Myzus Persicae* in iceberg lettuce. Within this, we had 2 objectives that we wanted to meet. The first was whether resistance was constitutionally expressed or induced. The second was to determine if age affects resistance.

In terms of our approach, we designed 5 possible experiments that related to our aim. Following this, we narrowed it down to the 3 most relevant experiments that we felt would answer our questions best. We also decided on these 3 because they demonstrated a range of experimental techniques which would provide us with different types of data to analyse. The experimental techniques that we decided to use were observation and EPG.

Our experiments involved plants that were 3 weeks and 6 weeks old. To plan when the experiments would be conducted, we took note of when they were sown. This gave us an indication of when to do the experiments. We kept a diary detailing what we were going to do on each date. We also produced a GANNT chart to ensure that we had a realistic and clear plan that was easy to follow.

Before we started the experiment, we ensured that we would each contribute equally and so have an equal share of the workload. We conducted 3 experiments which meant that each one of us could write up the method, results and discussion for one experiment, despite the fact that we completed all experimental work together.

Work Assignment

We each equally worked on the report. We wrote up the abstract, introduction, aims and objective and the conclusion together. We sat together and all contributed equally to these sections of the report. We had the task of writing up 1 experiment each.

- Experiment 1, including method, results and discussion was completed by Sonia.
- Experiment 2, including method, results and discussion was completed by Juweria.
- Experiment 3, including method, results and discussion was completed by Isabel.

We each contributed equally to the practical work. For experiment 1, this included observing and recording aphid behaviour on 1 resistant and 1 susceptible plant each. For experiment 2 and 3, this included making pins for the EPG machine; wiring the aphids to put on the plants; setting up the EPG machine and observing the progression of the EPG (making sure aphids were still on the plant; ensuring the connection was fine and making sure that traces were not off the scale).

For the analysis of our results for experiment 1, we discussed together what we felt the results concluded. Sonia calculated the means of our results and produced graphs based on them. For the analysis of experiment 2, we each analysed 2 traces – 1 resistant and 1 susceptible and calculated the relevant numerical figures. We produced the graphs for the experiment together and discussed the conclusions of the experiment, based on the results. For experiment 3, we each analysed two EPG traces and calculated the relevant numerical figures. We produced the graphs and discussed the conclusion together as a group.

Material and resources

In order to complete the project, one of the resources we needed was the assistance of Dr. Laura Vickers. This is because initially, we did not know how to use the EPG machines. Also, she taught us how to interpret the EPG traces as well as how to set up the EPG machines, including making pins and wiring up aphids. In addition to that, she gave us some background information and context about the aphids and the lettuce plants that proved useful to our project. Furthermore, other resources we required were access to laboratory equipment and access to the growth room. The cultivar of the resistant lettuce we used was LJ10224 and was the mapping parent line, provided by Dr. Paul Hand at Harper Adams University. The cultivar of susceptible plants we used was called Soleison from the company G's, provided by Ed Moorhouse. We also required *Myzus Persicae* (peach-potato aphids). Another resource we used was the laptops for research and for the analysis and write up of our experiments.

Experiment 1: Aphid Behaviours

Firstly, we decided to do an observation of how aphids behave when on both susceptible and resistant lettuce plants in order to get an indication of whether or not resistance is due to constitutionally expressed factors.

We each conducted an observation using a susceptible lettuce plant and an observation using a resistant lettuce plant – overall, observations were conducted on 3 susceptible plants and 3 resistant plants. We each conducted one observation at a time.

Initially, we decided on placing 10 aphids on each plant and observing their behaviours at each instance. However, we found that the lettuce leaves were fairly small, and therefore, it would be more appropriate to use 5 aphids instead - so that they are not overcrowded and uncomfortable. Using 5 aphids also made it easier to observe the aphid behaviours individually at each instance.

The way in which we structured the observations were as follows. The observation was conducted over 20 minutes, and observations of aphid behaviours were recorded at minute instances – every minute for 20 minutes. At these instances, percentages were calculated to represent the aphid behaviours at that point in time. For example, if at one instance 2 aphids were still and 3 were walking, the percentages would be: 40% still and 60% walking. In order to select the aphids to use, we used a thin paint brush dipped in water to slightly nudge the aphids. Some of the aphids did not respond, however, others did respond which was indicated by their movement. We selected the aphids that responded to this for our investigation. This was because the aphids that did not respond were likely to be old, and therefore not suitable to use in the observation as their behaviours may be down to their age rather than the nature of the plant itself.

Once the aphids were on the plant, we did not start the observation straight away – we gave them 10 minutes to be able to adjust to the new conditions of the plant, providing us with more representative data.

We did not use each aphid more than once because this could have affected the results obtained. For example, an aphid may have fed off the previous lettuce plant, affecting how it would behave on the next plant.

The behaviour categories that we used were still, walking, antennae forwards and antennae backwards. When an aphid is still, it is feeding from the plant, however, when and aphid is walking, it is not feeding. These 2 behaviours cannot occur at the same time and so are mutually exclusive. If an aphid has its antennae forwards, it is looking around and is cautious – it is not relaxed. However, when an aphid had its antennae backwards, it is happy and relaxed. Again, these 2 behaviours are mutually exclusive.

5 **Antennae Backwards**

6 **Antennae Forwards**

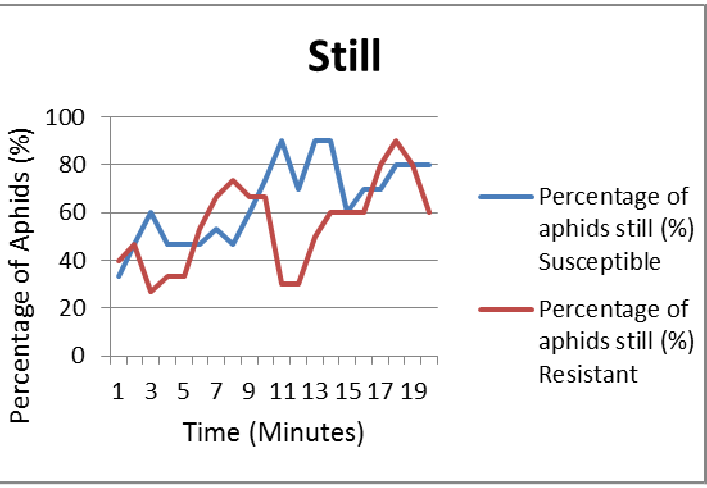
The independent variable of this investigation was the type of plant – resistant or susceptible. The dependent variable was the percentage of aphids showing the different behaviours (at each instance).

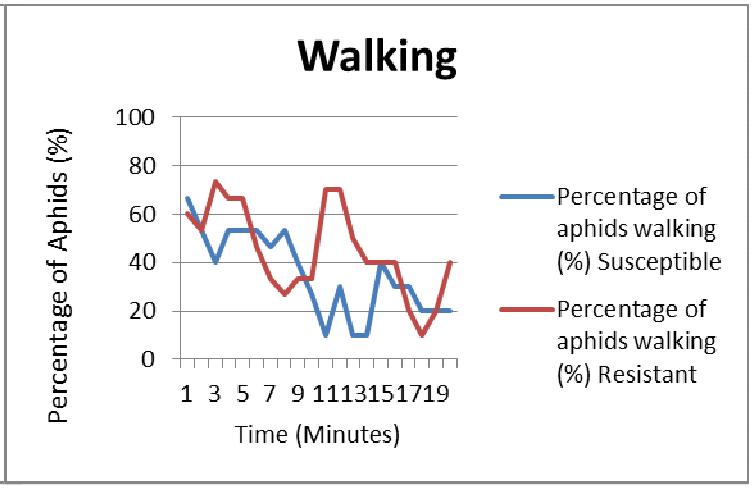
The factors that were kept the same (control variables) were that all the plants were the same age – all sown on the 25th of July; all the plants were grown in the same conditions;

the plants were all the same species (iceberg lettuce); each plant had 5 aphids on it and behaviours were recorded each minute for 20 minutes.

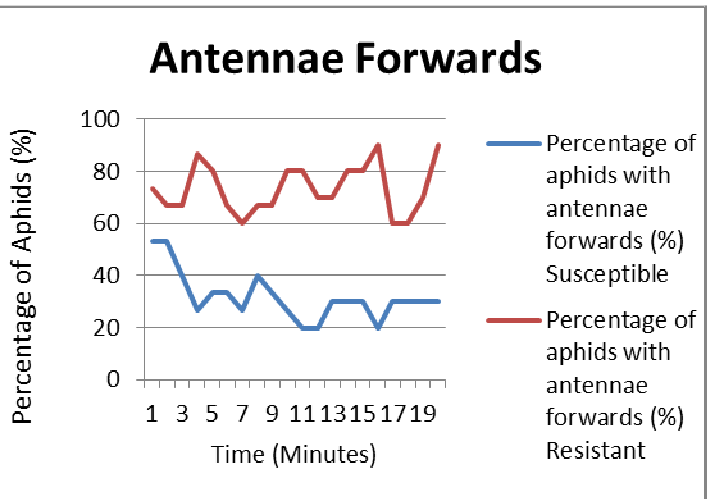
Once the observation was finished, we collated our data by calculating the mean values for the percentages of aphid behaviours at each instance.

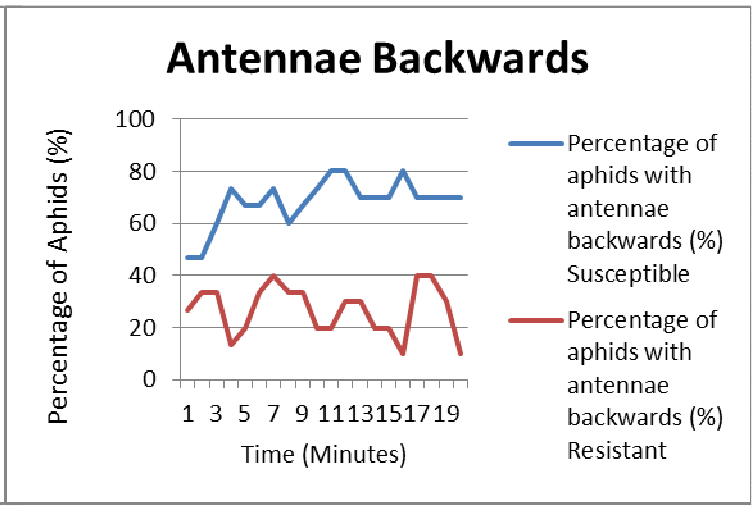
The results were as follows:





The above graphs show that in terms of staying still, generally, percentage of aphids for both types of plant increased. In terms of walking, generally percentage of aphids for both types of plant decreased. Despite this there were many fluctuations. There were fluctuations in the percentage of aphids staying still in both the susceptible and resistant plants at each instance. Hence, there were many fluctuations in the percentage of aphids walking in both the susceptible and resistant plants at each instance. Overall, there were more instances where a greater percentage of aphids were staying still on the susceptible plants. Similarly, there were more instances where a greater percentage of aphids were walking on the resistant plants. Despite this there is no clear considerable distinction between the results for resistant plants and susceptible plants.





The above graphs show that at every instance, there were a higher percentage of aphids with their antennae forward on the resistant plants than the susceptible plants. Hence at every instance, there were a higher percentage of aphids with their antennae backwards on the susceptible plants. Despite this, there were fluctuations in the data obtained within the 20 minutes.

In terms of the antennae, the results obtained were as expected. At all instances, a higher percentage of aphids on the resistant plants had their antennae forwards. This was most likely because a constitutionally expressed plant defence on the lettuce surface was causing resistance. This will have been causing the aphid to feel uncomfortable, not allowing it to relax – the leaf surface may have been the wrong texture, smell or taste for the aphids. Similarly, at all instances, a higher percentage of aphids on the susceptible plants had their antennae backwards. This was most likely due to the fact that there weren't any constitutionally expressed plant defences on the leaf surface of the susceptible lettuces that will have caused discomfort to the aphids – the conditions of the plants were appropriate for the aphids to be able to relax.

In terms of the "still" and "walking" behaviours, there were no considerable differences between the susceptible and resistant plants. This was possibly because the aphids were still getting used to the conditions of the plant. As well as this, on the resistant plants, the aphids may still have tried to penetrate the plant leaves with their stylets in order to see whether they could feed from the plant effectively or not, despite the constitutionally expressed plant defences on the leaf surface.

Overall, the results of this experiment suggest that on the resistant lettuces, there are plant defences on the surface that are constitutionally expressed. This finding was important as it allowed us to identify a type mechanism of resistance. However, as we do not know which specific type of constitutionally expressed resistance was responsible for the difference in aphid behaviour, future investigations aim to determine this – to identify the specific mechanism of resistance responsible.

Due to the indistinguishable results in terms of aphid movement, if this experiment was repeated in the future, I would give the aphids a larger gateway of time at the start of the experiment to allow them to adjust to the conditions of the lettuce plants (before starting the experiment).

I would also have used smaller intervals between instances (30 seconds). This is because the observation was instantaneous – the results didn't reflect the whole 20 minutes, but instead reflected the instances within the 20 minutes when observations were recorded. Using smaller intervals between instances will have provided data that is more representative of the 20 minutes.

A problem that we encountered when doing this experiment was that due to the small size of the lettuce leaves, there were occasions when the aphids fell off the leaves. We therefore had to use a thin paint brush to gently place them back on the plant. This may have caused the aphids discomfort, potentially affecting their behaviour (in terms of antennae position), hence potentially affecting the results of the experiment. In future, it would be ideal to use lettuce plant with larger and flatter leaf surface to avoid this issue.

Experiment 2: EPG comparing resistant and susceptible lettuces at 3 weeks

In this experiment, we were trying to find out as to whether the aphid is feeding from the resistant plant in comparison to the susceptible plans. Moreover, we used the EPG technique which allowed us to judge whether the plant defences of the resistant plant is constitutionally expressed or induced. The EPG technique required us to wire up an aphid on a thin wire and a metal rod in the soil of the plant to make an electrical circuit. This showed up on a computer in waveforms which we could analyse to see whether the aphid was penetrating its stylets into the plant or not penetrating, whether it was in pathway or if it was not feeding at all. We choose this technique to find answer our questions due to the fact that it allowed us to look in more detail their feeding habits and whether feeding from the resistant plant are the cause of the aphids not being able to reproduce.

Our first job in setting up the experiments was making the pins for the EPG machine. This was made from a small nail with a copper strip wrapped tightly around the top part of the nail. Then, we attached a very thin gold thread with a small silver paint at the bottom of the thread. The silver paint allowed us to attach it on the backs of the aphids so that electrical current could pass through the aphid, down to the metal rod which is connected to the computer to pick up the waves.

Secondly, when choosing the aphids we wanted to test on, we had two choices - there were some aphids which were on a cabbage plant and another batch of aphids which we contained in a container. We chose the aphids on the cabbage plant as we felt they were more ‘happy’ and less stressed than the aphids contained in a container. Thereof, we choose the 8 aphids and gently prodded them to retrieve their stylets from the cabbage plants. Then we carefully stuck them on to the pins with the silver paint then attaching them on to the EPG machine.

With the aphids attached, we had 8 plants in total which were just under three weeks old. We had 4 resistant plants under 4 channels and 4 susceptible plants under the other 4 channels. This allowed us to have a range of data instead of relying on one channel for each type of plant. Furthermore, it allowed us to be cautious that by having more channels, if something went wrong with the aphid we still have data from the other channels.

We began the experiment and let it run for four hours. In these four hours, we kept checking regularly to see if the aphid was still attached, whether it was on the plant, and if

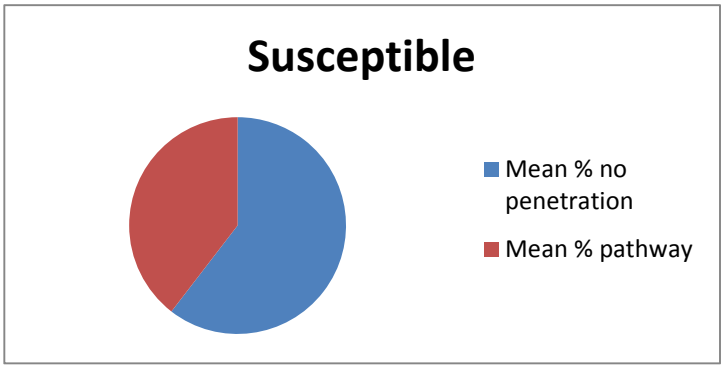
the voltage was not off the scale. By constantly checking, we hoped our results were as reliable as possible. In addition, we did a second run to again have a range of data that we could analyse.

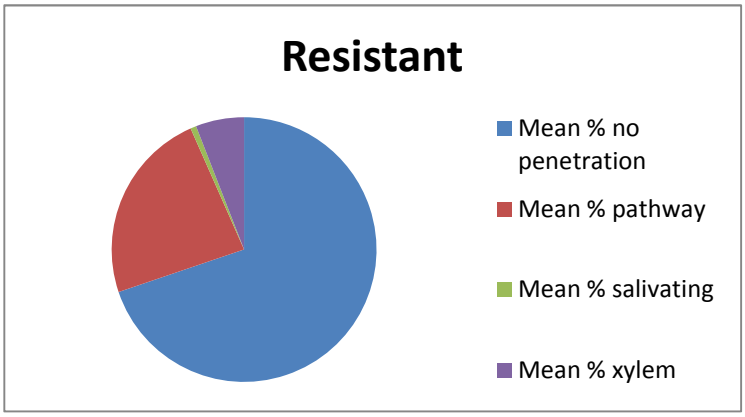
The independent variable in this experiment was the type of plants - which were susceptible and resistant. The control variables were the time the EPG machine was running (4 hours) and the age of the plants (3 weeks). The dependent variable for this experiment was the mean percentages of no penetrations, cell penetrations and pathway.

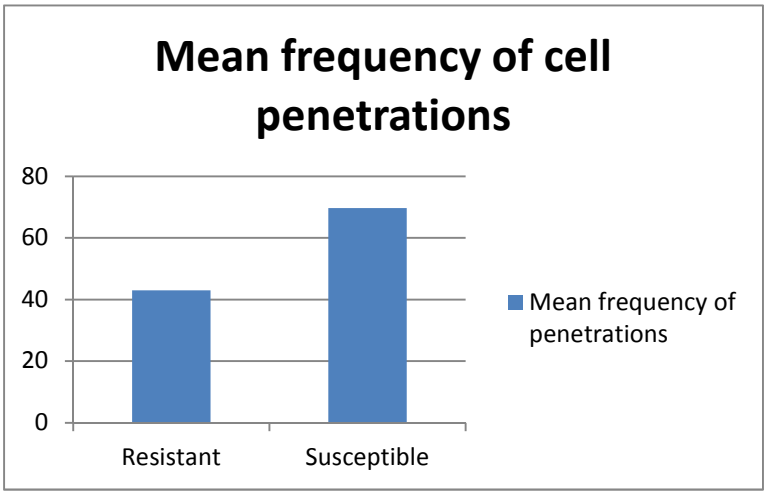
We analysed the EPG on software that which tells us the type of behaviour from the waveforms, the time it was and the voltage. This was represented on a grid which we then transferred onto Excel workbook to work out the time the aphid spent on the behaviours. Then we calculated a mean for all the results and then represented it on a pie chart. For the number of penetrations we decided to tally them as we were only looking for the number of times the aphid actually penetrated into the plant. This we represented it into a bar chart.

Our results are as follows:

After analysing our EPG traces and working out an overall mean, we realised that the susceptible plants had more mean percentage of no penetrations than the mean percentage for the pathway. On the other hand the resistant plants had more mean percentage of no penetration in comparison to mean percentage of pathway. Unlike the susceptible plant, the aphids on the resistant plants had salivated and fed from the xylem. Also, the resistant plant had more mean percentage of no penetrations than on the susceptible plants.







Furthermore, from finding the mean frequency of cell penetrations, the resistant plant had less than the susceptible plant.

From working out the mean, we realised that the susceptible plant had more mean percentage of no penetrations than mean percentage of pathway. In comparison to the resistant plant we expected to have more mean percentage of no penetrations. From this experiment, we believe that there were fewer penetrations in the resistant plant because there may have been more constitutionally expressed plant defences. This may include a thick waxy cuticle which may be hard for the aphid to insert its stylets. Another constitutionally expressed factor may be that the texture of the plant is not right for the aphid. It can be that the resistant plant is not giving off the right volatiles which can deter the aphid from feeding. Additionally, in the resistant plant the aphids salivated and fed from the xylem. From this, we drew the conclusions that perhaps the aphid salivated but did not ingest as it may not taste right or it may contain toxic (sucrose). In terms of the xylem feeding we believe after disliking what is contained in the phloem due to maybe the callous plug or the toxins present in the phloem. However, the fact that both susceptible and resistant plants did not have phloem feeding was unexpected. The aphids we used in our experiment may have already fed from the cabbage that they were previously on and hence did not give us the result we wanted. Therefore, in future, we would use starved aphids to give us a more reliable data such as phloem feeding in the susceptible plants.

Subsequently, through this technique, we realised that the experiment showed more of constitutionally expressed plant defences. This is because there were not that many penetrations, more salivation and more xylem feeding in the resistant plants than the susceptible plants. In addition, potentially there is a chance of there being induced expressed defences in the plants. However, this result came from one trace hence it is possible that it could be an anomaly.

<u>Experiment 3 - EPG comparing resistant plants of different ages</u>

The aim of this experiment was to determine whether resistance to *Myzus Persicae* is affected by age in resistant iceberg lettuce. To do this we compared the EPG traces of *Myzus Persicae* on resistant lettuce at 6 weeks, 5 days old (potted on the 4th July), to our previous traces of resistant lettuce at 3 weeks old (potted on the 25th July).

The equipment we used for this experiment was; an eight channel Electrical Penetration Graph (Fig. 1), 6 week 5 days old resistant iceberg lettuce plants, aphids (*Myzus Persicae*), and EPG pins. We also used an analysis programme for analysing the traces.[6]

Before setting up the EPG machine, we made the EPG pins (Fig. 2) in advance. This saved us time when attaching the aphids and setting up the machine. To make the pins, we used a nail and attached copper wire to the head with silver paint. We then attached 0.25mm thick gold wire to the copper wire with silver paint, and put a bead of silver paint on the end of the gold wire (this bead is where the pin attaches to the aphid's back). We used silver paint

as an adhesive as it is conductive, and in order for the EPG to work we needed the circuit to be complete. We took care when handling the silver paint to avoid spills and kept a lid on it to prevent it evaporating. We also handled tweezers and razor blades carefully to avoid injury. As it was likely that some of the pins would break or not work when in the EPG, we made more pins than needed to compensate for this and overcome this problem.

In order to make it easier to attach the pin to the aphid, we chose larger, adult aphids. To choose which of these to use for the EPG we used a paintbrush to gently nudge them, and if they responded by moving away rather than staying still we chose them.

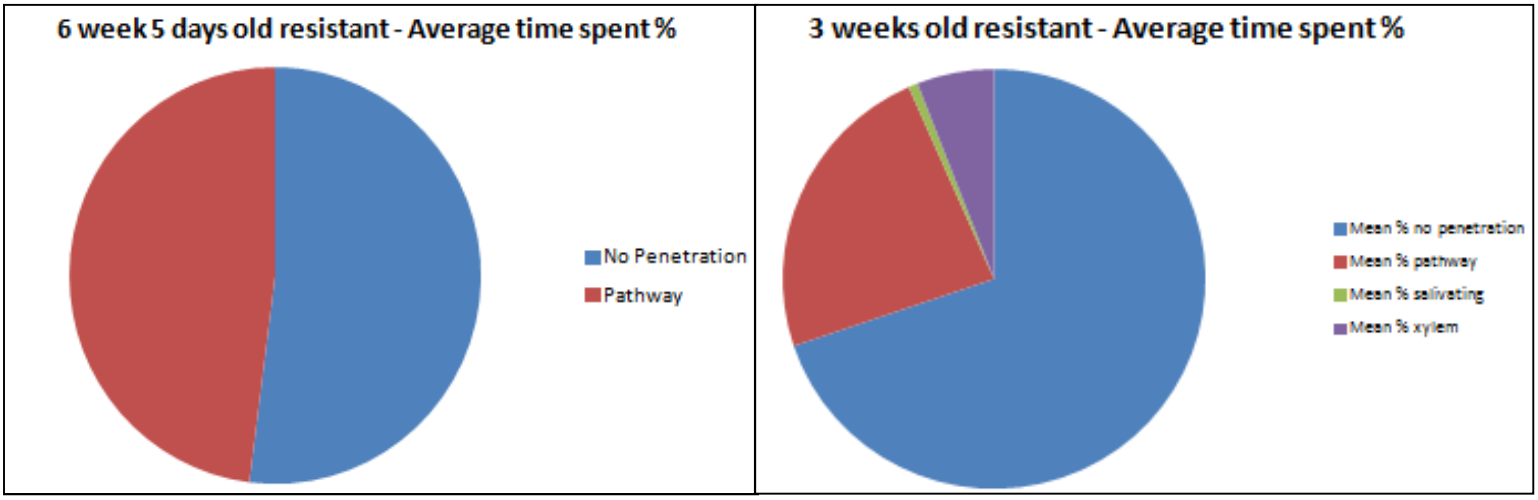
To set up the machine we first attached the pins to the aphids, by putting silver paint on the bead at the end of the pin, and gluing it to the aphid's back. It was necessary to make sure the bead was in the correct position and would not interfere with the aphid's ability to walk around and feed. We used a paintbrush dipped in water to handle the aphids as they are soft bodied, and we did not want to harm them. Once the aphids were attached to the pins (Fig. 3), we inserted the nail part of the pin into the EPG, and placed the aphid on the plant leaf, making sure the gold wire was positioned so the aphid could move around, but wouldn't fall off. We then set the EPG to run for four hours and turned it on. Whilst the machine was running, we made sure to check on the aphids regularly, and also adjust the dial if the voltage went off the scale. Once the four hours were finished, we repeated the experiment with new aphids for another four hours.

We used an eight channel EPG, and used 6 week 5 days old plants in each channel. In total we achieved sixteen, four hour traces, but not all of these were able to be analysed due to aphids falling off the plant or a weak connection in the circuit. We ended up with six useable traces, and each analysed two.

The control variables we kept the same were the conditions that the plants were grown in (same compost, temperature, light exposure and water); also the aphids were all genetic clones of each other and previously feeding on the same plant. The independent variable was the age of the plants (we compared our traces from experiment 3, at 6 weeks 5 days, to our previous traces of resistant lettuce from experiment 2, at 3 weeks.). The dependent variables we recorded were percentage time spent not penetrating, in pathway, salivating, phloem feeding and xylem feeding, and the number of cell penetrations made during the four hours.

Once the EPG machine was finished, we each analysed two traces and from this, calculated the percentage time spent doing each activity (no penetration, pathway, salivating, phloem feeding, xylem feeding), and totalled the number of cell penetrations made. The cell penetrations had to occur whilst the aphid was in pathway. We then combined all our results to calculate a mean. When calculating the mean we found that we had an anomalous result, and to overcome this we did not include this data in the mean.

Our results are as follows:

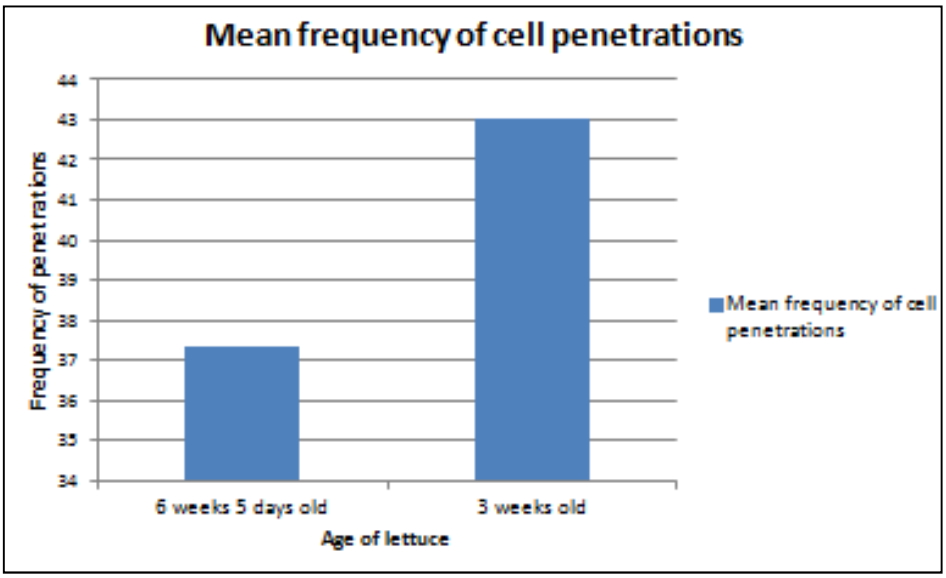


We chose to display this data in a pie chart in order to make it clear and easy to compare the percentages from each age. From these pie charts, we can see that at 6 weeks, the aphids spent a bigger percentage of time in pathway, and a smaller percentage of time not penetrating the plant, than at 3 weeks old. This suggests that aphids preferred the plant at 6 weeks as they were in the plant for more time. This could be due to the aphids preferring the texture, smell or taste of the older plants, so they are probing more in order to feed. This may suggest that the younger plants possibly have stronger constitutionally expressed defences than the older plants, such as a thicker cuticle or they may give off gases (volatiles) that the insects do not like.

However, we see no phloem feeding at either age, so this may mean that there is also resistance inside the plant, for example, a blockage of the phloem by a callose plug, or another induced defence. We do see xylem feeding at three weeks; this may be due to the aphid being unable to locate or access the phloem, or could be an anomaly as xylem feeding was only seen on one trace.

These results suggest that age does affect resistance, and that younger plants have stronger resistance to *Myzus Persicae* through stronger or more numerous constitutionally expressed defences. This may be because younger plants are more vulnerable, so need more protection than older plants. The aphids spent significantly less time in the lettuce at three weeks old compared to six weeks old, so may not have wanted to probe and try to feed from the plants. This could be because the plants are still very young, still developing and have not undergone as much photosynthesis, and the aphids simply prefer a bigger plant due to more amino acids or a better taste – not due to more defences in younger plants.

Also, the aphids may not be used to feeding on younger plants, as at 3 weeks old lettuce plants are not typically in fields yet.

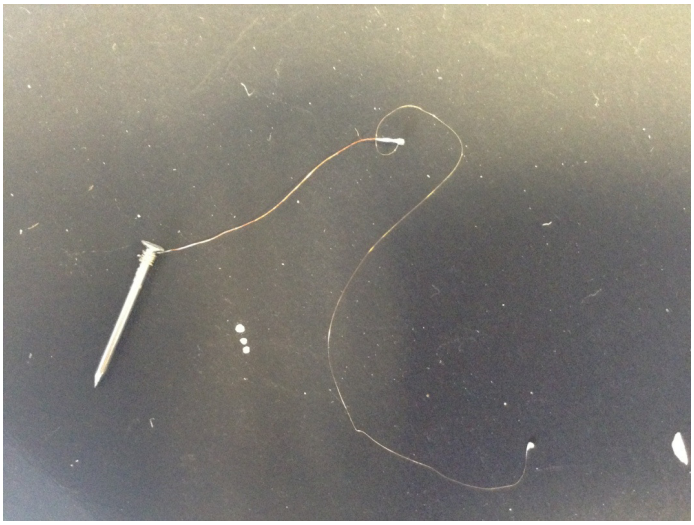


We chose a bar chart to present these results as we felt it would be the easiest way to show the difference in number of cell penetrations. This bar chart shows that despite spending less time within the plant (pathway) at 3 weeks, during this time there were significantly more cell penetrations. This could be because once they were in the plant the aphids found it difficult to locate the phloem so made more penetrations in an attempt to find it. This is possibly because there is no gradient to follow as it is yet to be set up in the younger plants, whereas it is in the older ones. However, we would assume we would have seen some phloem feeding in the older plants if this was the only case, so there is likely to be a defence in the phloem in all ages of resistant lettuce, and perhaps stronger constitutionally expressed defences, such as wrong taste, texture or smell, in the younger ones.

If I were to do this experiment again, I would run the EPG machine more times in order to obtain many more traces. This would give us a more reliable mean and help to identify any anomalies. Also, the reason for not seeing any phloem feeding may be because the aphids had already been feeding on another plant before they were moved to the lettuces, so in a repeat of the experiment I could starve the aphids before using them in the experiment. It may also be useful to run the EPG machine for longer to see whether the aphid's behaviour would change over time.

Fig. 1 – EPG machine with 3 week old plants attached

Fig. 2 – EPG pin



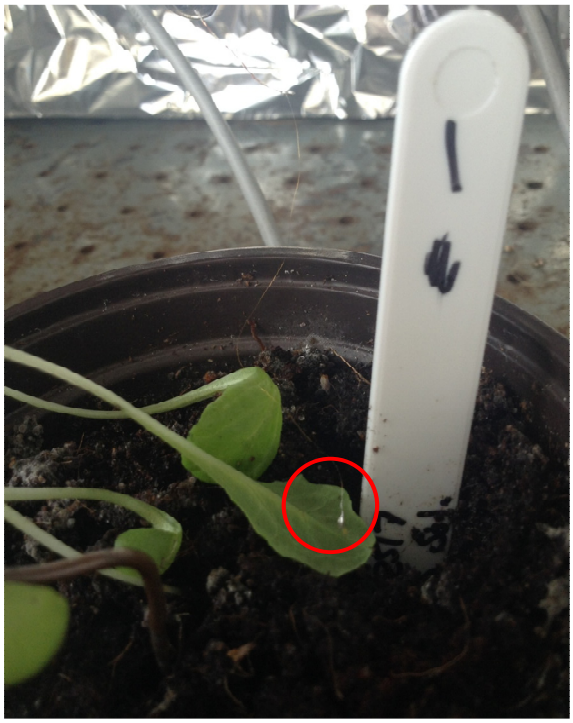


Fig. 3 – Aphid attached to EPG pin

Conclusion

From the experiments we have conducted, we have concluded that the mechanism of resistance in our resistant cultivar of lettuce is likely to be constitutionally expressed (such as wrong leaf surface texture or undesirable smell or taste – further investigation would be required to determine which) and is negatively affected by ageing. Experiments 2 and 3 suggested that there may possibly be induced defences in the lettuce. The impacts of our conclusion to the food production industry are that we would suggest to farmers who grow lettuce that growing resistant varieties would be more economically viable, because of the reduced need to use pesticides. It would be useful to invest in further research to be able to implement these natural mechanisms of resistance in other varieties of lettuce. We also suggest that farmers reduce the use of pesticides on younger lettuce plants because they seem to have stronger natural defences against aphids.

Throughout our project, we learnt practical laboratory skills such as the usage of an EPG machine. We also learnt a lot of theory about aphid behaviour and plant defences- this assisted us when drawing conclusions. Other skills we learnt included organisation, planning, independence, teamwork, time management and written and verbal communication. We gained experience of working in a lab environment and completing an independent research task. We also demonstrated a range of creative abilities throughout the duration of our project. For example, regarding the EPG traces, we decided how to interpret the data and visually display it in a graph which was rich in data but clear to understand. We also designed our own observation and came up with a method to obtain and record results. We were also creative when drawing conclusions because we looked at the data from multiple perspectives before opting for the most logical ones.

References

[1] Vickers, Project Description for STEM Summer Placement, University of Birmingham, 2014

[2] Anon. (Unknown). *What are the advantages and disadvantages of pesticides?.* Available: http://wiki.answers.com/Q/What_are_the_advantages_and_disadvantages_of_pesticides. Last accessed 15th Aug 2014.

[3]Vickers, Induction Talk, University of Birmingham, 11/08/2014

[4] Brouwer, http://www.epgsystems.eu/, Accessed 15/08/2014

[5] http://www.forestryimages.org/images/768x512/1549196.jpg

[6] https://macrophotoray.files.wordpress.com/2011/04/mg_8331.jpg

[7] http://www.epgsystems.eu/

Appendix

Silver paint risk assessment

2. HAZARDS IDENTIFICATION

Classification R10
Xi; R36
R66, R67
N; R50
:
The product is classified as dangerous according to Directive 1999/45/EC and its amendments.
Physical/chemical hazards : Flammable.
Human health hazards : Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapours may cause drowsiness and dizziness.
Environmental hazards : Very toxic to aquatic organisms.
See section 11 for more detailed information on health effects and symptoms.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Substance/preparation Preparation
Occupational exposure limits, if available, are listed in section 8.
:
CAS
number
Ingredient name % EC number Classification
SILVER 7440-22-4 35 - 65 231-131-3 N; R50 [1] [2]
2-methoxy-1-methylethyl acetate 108-65-6 10 - 30 203-603-9 R10
Xi; R36
[1] [2]
n-butyl acetate 123-86-4 10 - 30 204-658-1 R10
R66, R67
[1] [2]
See section 16 for the full text of the R-phrases
declared above
[1] Substance classified with a health or environmental hazard
[2] Substance with a workplace exposure limit
There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4.

First-aid measures
Move exposed person to fresh air. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. Keep person warm and at rest. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.
Inhalation :

FIRST AID MEASURES

Date of issue/Date of revision
: 6/23/2009. ***1/7***
Product: Electronically conductive paint - silver loaded
Article Number: RS 186-3593, 186-3600
Application: Used for producing or repairing PCB track.
Supplier: RS Components Ltd,
Birchington Road,
Corby, Northants,
NN17 9RS,
UK
Tel: +44 (0) 1536 402888 (8am to 8pm)
Fax: +44 (0) 1536 401588
Email: technical.help@rs-components.com

CP0225 v4.1 RS 186-3593, 186-3600 RS REACh revision date 01/07/09

Electrically Conductive Paint

4. FIRST AID MEASURES

Wash out mouth with water. Remove dentures if any. Move exposed person to fresh air. Keep person warm and at rest. If material has been swallowed and the exposed person is conscious, give small quantities of water to drink. Stop if the exposed person feels sick as vomiting may be dangerous. Do not induce vomiting unless directed to do so by medical personnel. If vomiting occurs, the head should be kept low so that vomit does not enter the lungs. Get medical attention if adverse health effects persist or are severe. Never give anything by mouth to an unconscious person. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

Skin contact

Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention.

Wash skin thoroughly with soap and water or use recognised skin cleanser. Remove contaminated clothing and shoes. Obtain medical attention if symptoms occur. Wash clothing before reuse. Clean shoes thoroughly before reuse.

Notes to physician No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

Ingestion
Eye contact
:
:
:
:

See section 11 for more detailed information on health effects and symptoms.

Protection of first-aiders : No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

5. FIRE-FIGHTING MEASURES

Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool. This material is very toxic to aquatic organisms. Fire water contaminated with this material must be contained and prevented from being discharged to any waterway, sewer or drain.

Hazardous thermal decomposition products
Special exposure hazards

Decomposition products may include the following materials:
carbon dioxide
carbon monoxide
metal oxide/oxides

Flammable liquid. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion. The vapour/gas is heavier than air and will spread along the ground. Vapours may accumulate in low or confined areas or travel a considerable distance to a source of ignition and flash back. Runoff to sewer may create fire or explosion hazard.

Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Special protective equipment for fire-fighters

Use dry chemical, CO_2, water spray (fog) or foam.

Extinguishing media
:
:
:

Do not use water jet.

Suitable :

Not suitable :
Environmental precautions
Personal precautions
Stop leak if without risk. Move containers from spill area. Approach the release from upwind. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13). Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor. Contaminated absorbent material may pose the same hazard as the spilt product. Note: see section 1 for emergency contact information and section 13 for waste disposal.

6. ACCIDENTAL RELEASE MEASURES

:
: No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilt material. Shut off all ignition sources. No flares, smoking or flames in hazard area. Avoid breathing vapour or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
Avoid dispersal of spilt material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air). Water polluting material. May be harmful to the environment if released in large quantities.
Large spill :
Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Use spark-proof tools and explosion-proof equipment. Dispose of via a licensed waste disposal contractor.
Small spill :
Methods for cleaning up
Date of issue/Date of revision
: 6/23/2009. ***2/7***

Handling

HANDLING AND STORAGE

Storage

7.

Put on appropriate personal protective equipment (see section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Do not breathe vapour or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Avoid release to the environment. Refer to special instructions/safety data sheet. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Keep in the original container or an approved alternative made from a compatible material, kept tightly closed when not in use. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment. Use non-sparking tools. Take precautionary measures against electrostatic discharges. To avoid fire or explosion, dissipate static electricity during transfer by earthing and bonding containers and equipment before transferring material. Empty containers retain product residue and can be hazardous. Do not reuse container.
Packaging materials
Store in accordance with local regulations. Store in a segregated and approved area. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Eliminate all ignition sources. Separate from oxidizing materials. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabelled containers. Use appropriate containment to avoid environmental contamination.
:
:
Recommended : Use original container.
Ingredient name Occupational exposure limits
Recommended monitoring procedures
If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment. Reference should be made to European Standard EN 689 for methods for the assessment of exposure by inhalation to chemical agents and national

guidance documents for methods for the determination of hazardous substances.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Hand protection

Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirato

Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this necessary.

Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

Eye protection

Respiratory protection :

:

:

:

SILVER **EH40-WEL (United Kingdom (UK), 8/2007).**

WEL 8 hrs limit: 0.1 mg/m³ 8 hour(s).

2-methoxy-1-methylethyl acetate **EH40-WEL (United Kingdom (UK), 8/2007). Skin**

WEL 15 min limit: 548 mg/m³ 15 minute(s).

WEL 15 min limit: 100 ppm 15 minute(s).

WEL 8 hrs limit: 274 mg/m³ 8 hour(s).

WEL 8 hrs limit: 50 ppm 8 hour(s).

n-butyl acetate **EH40-WEL (United Kingdom (UK), 8/2007).**

WEL 15 min limit: 966 mg/m³ 15 minute(s).

WEL 15 min limit: 200 ppm 15 minute(s).

WEL 8 hrs limit: 724 mg/m³ 8 hour(s).

WEL 8 hrs limit: 150 ppm 8 hour(s).

Skin protection Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

:

Environmental exposure controls

: Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable leve

Butyl Acetate Risk Assessment:

ACTIVITY/METHOD:	USING BUTYL ACETATE
WHAT IS THE RISK?:	POTENTIAL CONTACT WITH SKIN AND EYES, POTENTIAL SPILLAGES, IS A FLAMMABLE CHEMICAL

PERSONS AT RISK:	PEOPLE USING THE CHEMICAL

WORKPLACE						
WHERE is the activity to be carried out?						
In the laboratory						
WHAT are the hazards? (answer YES or NO in ALL boxes)						
✓	Slips, trips, falls		Lighting			
	Falling Objects		Ventilation			
	Obstructions/Protrusions		Temperature		Pressure/vacuum	
	Electrical Equipment		Vibration		Chemical	
✓	Fire/Explosion		Particles and Dust		Biological	
	Other	Harm caused to skin or eyes.				

EXISTING CONTROL MEASURES
• Ensure there are no flames present where the Butyl Acetate is being handled
• Handle with care to avoid spillages
• Should a spillage occur, clean up a soon as possible
• Handle with care to avoid contact with skin or eyes
• Use a pipette to avoid contact with skin
• Keep the lid on the jar to avoid spillages
• Should contact with skin occur, run under cold water as soon as possible

INITIAL ASSESSMENT OF OVERALL RISK	LOW	✓	MEDIUM		HIGH	

Project Presentation Feedback Forms

Presentation Feedback

Student Name(s): ISABEL, JULIERIA & SONIA

1) Were the following sections in the presentation clear;

A, Description of methods

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

B, Communication of results

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

C, Conclusion

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

2) What changes would you suggest be made to the presentation?

Very impressive presentation !

3) How did the speaker(s) respond to questions?

Student 1: V. Good

Student 2: V. Good

Student 3: V. Good

Thank you for you time

Presentation Feedback

Student Name(s): Isabel Pearson (1)
Juweria Faraz (2)
Sonia Dewi (3)

1) Were the following sections in the presentation clear;

A, Description of methods

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

B, Communication of results

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

C, Conclusion

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

2) What changes would you suggest be made to the presentation?

- more images (especially in introduction – for example an image of an optical – not everyone has seen them)
- take a little more time with a central graph
- it was very good – much better than many university undergraduates ☺

3) How did the speaker(s) respond to questions?

Student 1: excellent ++

Student 2: excellent ++

Student 3: excellent ++

Thank you for you time

no problem

Presentation Feedback

Student Name(s):

ISABEL, JUNGRA, SONIA.

1) Were the following sections in the presentation clear;

A, Description of methods

Very clear	Clear	Reasonable	Unclear	Very unclear
		✓		

B, Communication of results

Very clear	Clear	Reasonable	Unclear	Very unclear
			✓	

C, Conclusion

Very clear	Clear	Reasonable	Unclear	Very unclear
	✓			

2) What changes would you suggest be made to the presentation?

SPEAK LESS QUICKLY.
CONSIDER NOT USING CUE-CARDS.
TALK THROUGH FIGURES.

3) How did the speaker(s) respond to questions?

Student 1: CLEARLY AND CLEARLY THEIR WORK

Student 2:

Student 3:

Thank you for you time

Presentation Feedback

Student Name(s):

Isabel Pearson
Sonia Dari
Juweria Farah

1) Were the following sections in the presentation clear;

A, Description of methods

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

B, Communication of results

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

C, Conclusion

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

2) What changes would you suggest be made to the presentation?

slow down slightly whilst presenting, otherwise excellent.

3) How did the speaker(s) respond to questions?

Student 1: All three stood up well to questions and provided thoughtful responses

Student 2:

Student 3:

Thank you for you time

Presentation Feedback

Student Name(s): Isobel (1)
Juweria (2)
Sonia (3)

1) Were the following sections in the presentation clear;

A, Description of methods

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

B, Communication of results

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

C, Conclusion

Very clear	Clear	Reasonable	Unclear	Very unclear
✓				

2) What changes would you suggest be made to the presentation?

Introduced lettuce a little more, maybe diagrams to explain phloem, xylem etc and diagram showing EPG as well – ie/ showing it as a circuit

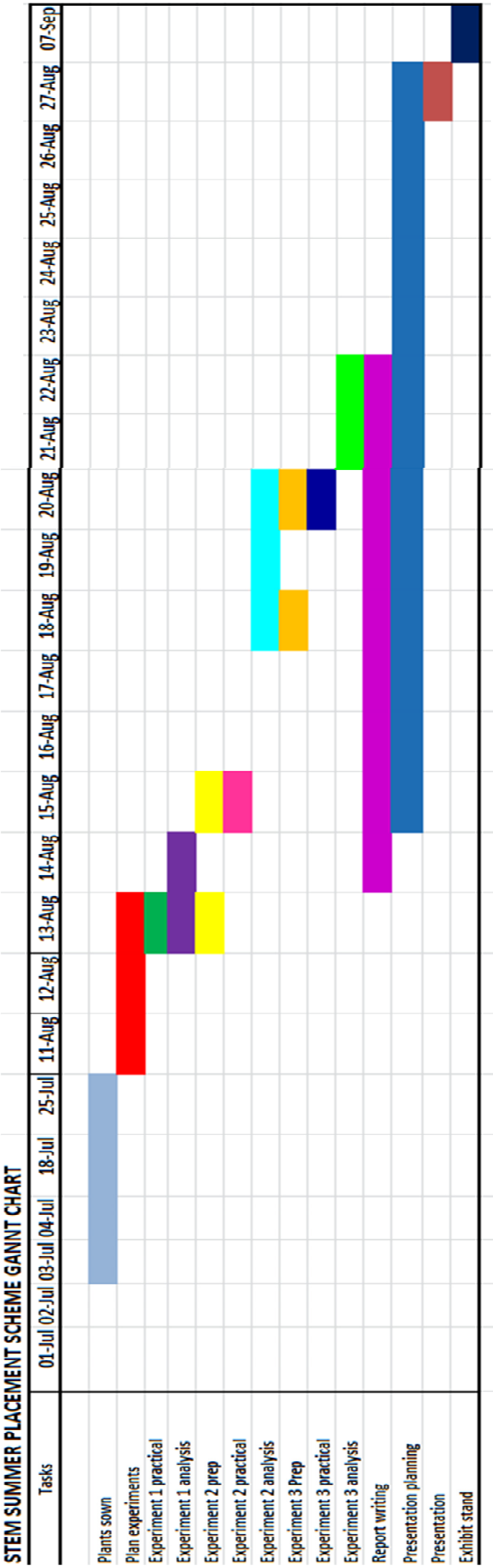
3) How did the speaker(s) respond to questions?

Student 1: Very well
Student 2: Very well
Student 3: Very well

– you had some difficult questions and handled them very well. All took turns, talk was a pleasure to listen to!

Thank you for you time

GANNT chart


STEM SUMMER PLACEMENT SCHEME GANNT CHART
Tasks
01-Jul
02-Jul
03-Jul
04-Jul
18-Jul
25-Jul
11-Aug
12-Aug
13-Aug
14-Aug
15-Aug
16-Aug
17-Aug
18-Aug
19-Aug
20-Aug
21-Aug
22-Aug
23-Aug
24-Aug
25-Aug
26-Aug
27-Aug
07-Sep
Plants sown
Plan experiments
Experiment 1 practical
Experiment 1 analysis
Experiment 2 prep
Experiment 2 practical
Experiment 2 analysis
Experiment 3 Prep
Experiment 3 practical
Experiment 3 analysis
Report writing
Presentation planning
Presentation
Exhibit stand