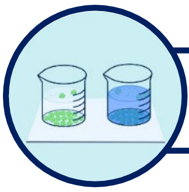
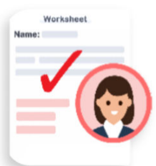
Photosynthesis Inhibitor Investigation



Photosynthesis Inhibitor Investigation

Table of Content



Notes for teachers
- Scan the QR code to get the electronic files.
- Teachers are strongly encouraged to adapt and modify these resources as necessary.

Innovations in Biology Investigations

Photosynthesis Inhibitor Investigation

Overview

- This *Photosynthesis Inhibitor Investigation* is about determining the mode of action of different herbicides.
- Students perform the leaf flotation method (Cookson & Price, 1982) to study the effect of herbicides on the photosynthesis of leaf discs (Hill & Steucek, 1985; Zemedkun et al., 2019).
- Students are given the opportunity to design and carry out experiments in which they consider the accuracy and reliability of methods of measuring the dependent variable, pooling class data, and the limitations of the methods in determining the mode of action of the herbicides.

Teaching Plan & Key Features

Prerequisite knowledge (scientific ideas)

- The process of photosynthesis
- The relationship between photosynthesis and respiration

Lesson	Lesson sequence	Duration (mins)	Resources
Stage ❶ Preparing for the investigation • Students read information to familiarise themselves with the background of the investigation (*Reading Materials*). • Student questions about the *Reading Materials* are addressed in class (*Diagnostic Assessment*).			
Before Lesson 1	• The teacher distributes *Worksheet 1* for students to complete at home for them to be familiar with the background of the investigation. • The teacher collects student questions about the investigation context using a *Google Form*.		*Worksheet 1*
1	• The teacher addresses student questions about the investigation context in a *Google Form*.	40	Student Samples 1
Stage ❷ Designing the investigation • Students physically interact with the materials and apparatuses before designing the investigation.			
2	• The teacher discusses with students questions related to their experimental designs. • The teacher provides students with the laboratory manual for preparation at home.	40	Teacher Notes 1
Stage ❸ Carrying out the investigation • Students use cameras to record data (*Digital Tool*).			
3	• Teacher asks questions to help students connect their lab experience and related ideas/scientific inquiry skills. • Students carry out the investigation.	40	Laboratory Manual
Stage ❹ Explaining and evaluating data • Students use data to deduce the mode of action of the herbicides (**Problem Solving Task**). • Students identify the limitations of the data collected in answering the investigation question.			
Before Lesson 4	• Students complete data reporting and analysis at home. • The teacher collects and marks student responses.		Teacher Notes 2
4	• The teacher provides feedback on students' performance related to data reporting and analysis.	40	Teacher Notes 2

Important Notes

- Students should wear safety goggles and lab coats during the experiment.
- Students should avoid skin contact with the solutions with herbicides.

Instructional Materials

Stage ❶ Preparing for the investigation

Student Worksheet 1

Notes for teachers

- Teachers can distribute *Worksheet 1* and instruct students to read the background information related to the investigation at home.
- Teachers collect student questions about the investigation using a *Google Form*.
- Scan the QR code to get a copy of the *Google Form*.

Task 1

- Read the following information and source materials in the *Data File*.

Scenario

Herbicides, also called weedkillers, are substances used to manipulate or control unwanted plants (or weeds). Different herbicides have different modes of action based on the mechanism by which the herbicide controls susceptible plants. Identifying this mode of action is important for selecting the right herbicide for each crop.

In this investigation, you will collect data to determine the modes of action of three herbicides. Read the information in the *Data File* to familiarise yourself with the context. You will use your biological knowledge of photosynthesis and the design of valid and reliable experiments, as well as the information in the *Data File* to complete this investigation.

Complete the *Google Form* after you have read the *Data File*.

Questions in the Google Form

(a) Self-assess your understanding about the information in the *Data File*.
- 0 – I don't get it.
- 1 – I kind of get it.
- 2 – I get it, but I need help to explain it.
- 3 – I get it, and I can explain it.
- 4 – I get it, and I can teach it to my friends.

Students self-assess their understanding of the reading materials and raise questions. Teachers can then address students' difficulties based on their responses.

(b) List *at least* one question you have about the investigation.

Data File

Your biology teacher asks you to read the following source materials to prepare yourself for designing an investigation related to the study of herbicides.

Source 1: Mode of action of herbicides

Herbicides are chemicals that prevent or stop the normal growth and development of weeds. Herbicides can increase crop yield. However, improper use can lead to crop inquiry, herbicide-resistant weeds, health risks, and environmental damage. Herbicides have different modes of action based on the mechanism by which the herbicide disrupts the normal growth and development of susceptible plants. Three common modes of action can be distinguished:

(1) Cell division inhibitors

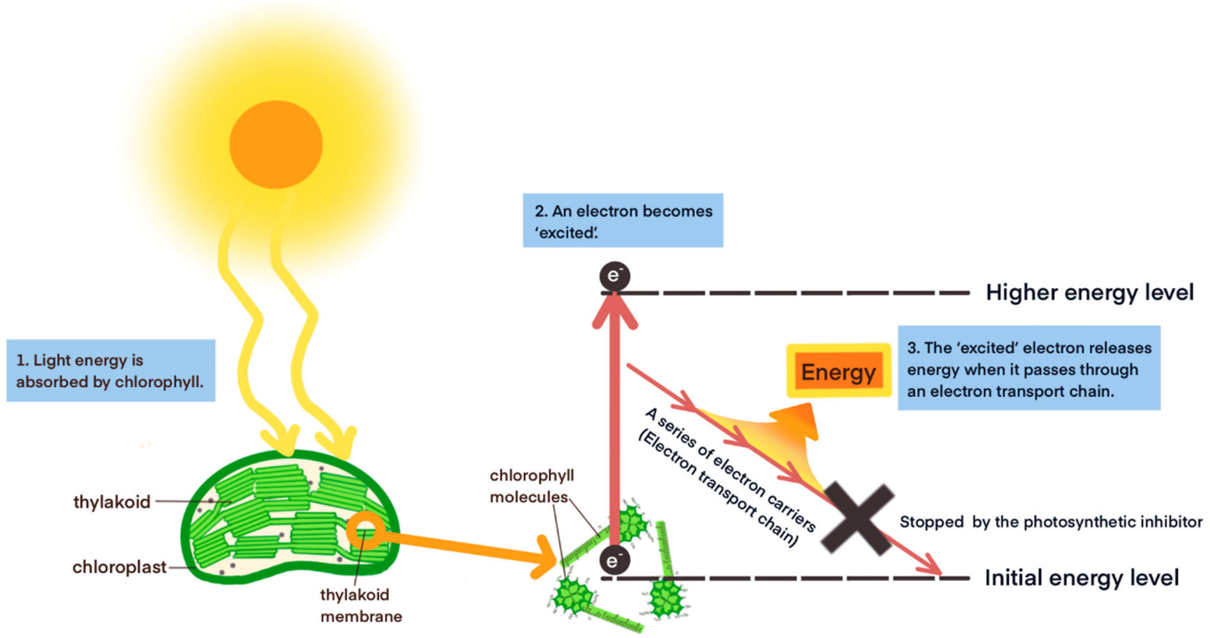
- This group of herbicides inhibits cell division and new growth. For example, some herbicides interfere with the process that leads to the correct arrangement of chromosomes during cell division. This group of herbicides can stop cell division at the root and root tips.

(2) Photosynthesis inhibitors

- These herbicides inhibit photosynthetic pathways. For example, some herbicides bind to an electron carrier involved in the light-dependent stage of photosynthesis. This interrupts the electron transport chain and thus the production of NADPH and ATP. These herbicides are often used when the weed has already germinated and developed photosynthetic activity.

(3) Synthetic plant growth regulators

- This group of herbicides mimics plant growth regulators. For example, some herbicides mimic the action of auxins. When these herbicides are applied to the leaves, they are transported to the meristems and cause uncontrolled growth. Other longer-term effects include leaf discolouration.



Source 2: Testing for photosynthesis inhibitors

What is the leaf flotation method?

- The leaf disc flotation method is a simple and quick way to identify photosynthesis inhibitors using leaf discs. If a photosynthesis inhibitor is present in the test solution, oxygen production is slowed down or stopped because the process of photosynthesis is inhibited, and the leaf discs do not float/float more slowly.

How is the leaf flotation method performed?

- Leaf discs are punched from fully expanded leaves. The leaf discs floating on the test solutions in a beaker are infiltrated under vacuum for 5 minutes to remove the air in the air spaces of the leaf discs.

Scan the QR code to see how leaf discs are vacuumed.

The beaker is then illuminated with a light source.

How can we compare the inhibitory effect of different photosynthesis inhibitors?

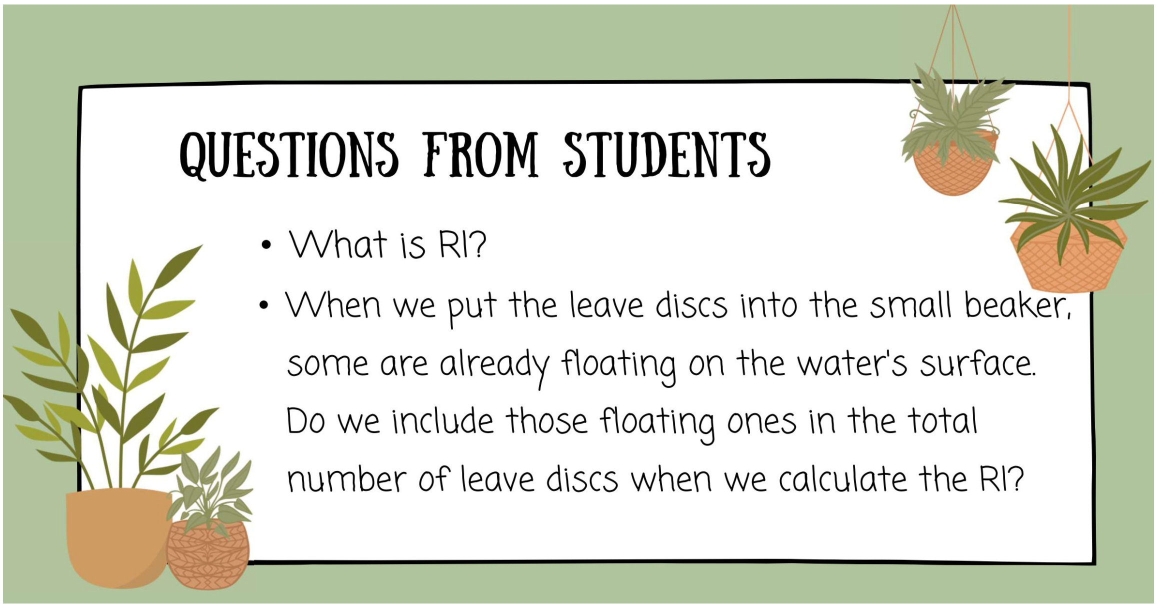
- The time it takes for a leaf disc to float to the surface of the test solution is an indicator of the photosynthesis rate of that leaf disc. As there are individual differences between leaf discs, some leaf discs float faster, while others float later.

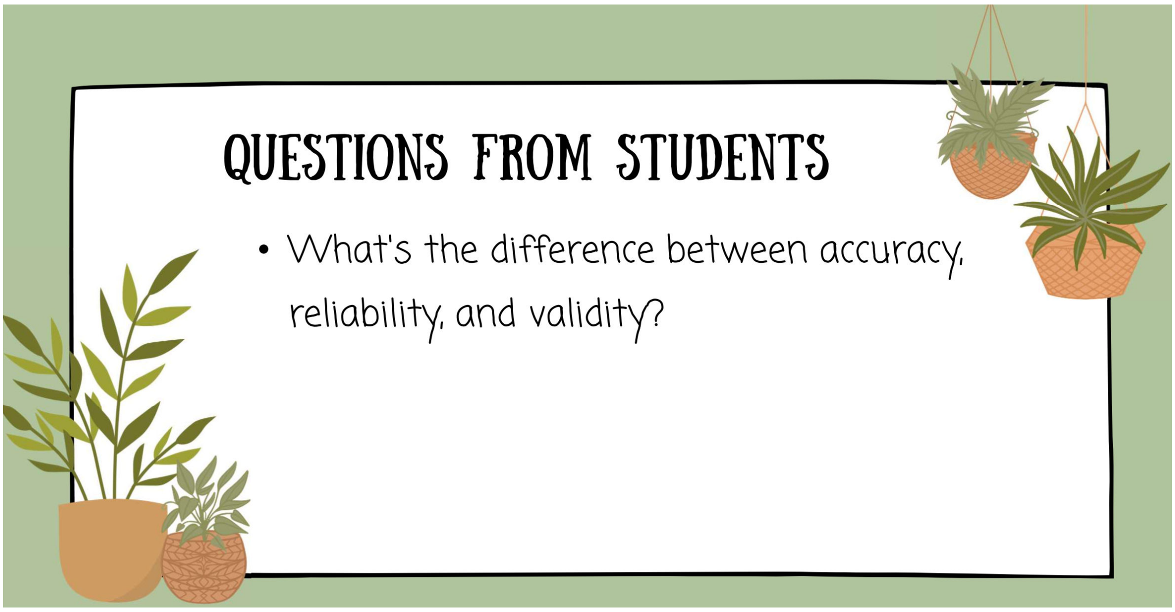
Scan the QR code to see what happens to leaf discs under light illumination.

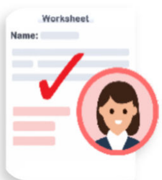
- The time that passes until 50% of the leaf discs float is called **ET50**. At this point, 50% of the leaf discs are floating. The time it takes for half of the leaf discs to float to the surface of the medium can be compared with that of the control. This ratio is called the **retardation index (RI)**.

How can the validity and reliability of the method be improved?

- All treatments should be repeated. Each replicate should consist of 10 or more leaf discs. The experiment should include a control to which no photosynthesis inhibitor is added.



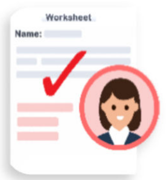




Notes for teachers

- Teachers can address students' questions about the investigation context.
- The examples of student questions show that students struggle to understand certain terms related to scientific inquiry. Instead of telling students the definitions of the terms, teachers can use examples related to the present investigation to explain the ideas.

Teacher Notes 1



Notes for teachers

- After addressing the student questions, teachers can show students the materials and apparatuses to facilitate their design. See the *Supplementary Resource* section for a list of materials.
- There are some questions that teachers may use to guide students in thinking about or assessing the scientific inquiry skills related to experimental designs.
- Some student work samples are shown below to illustrate possible student thinking to some questions.

Task 2

Scenario

Herbicides, also called weedkillers, are substances used to manipulate or control unwanted plants (or weeds). Different herbicides have different modes of action based on the mechanism by which the herbicide controls susceptible plants. Identifying the mode of action of herbicides is important for selecting the right herbicide for each crop.

In this investigation, you will investigate the effect of three different herbicides on the photosynthesis of leaf discs. This information can help you determine the mode of action of the three herbicides.

You are given the following materials:

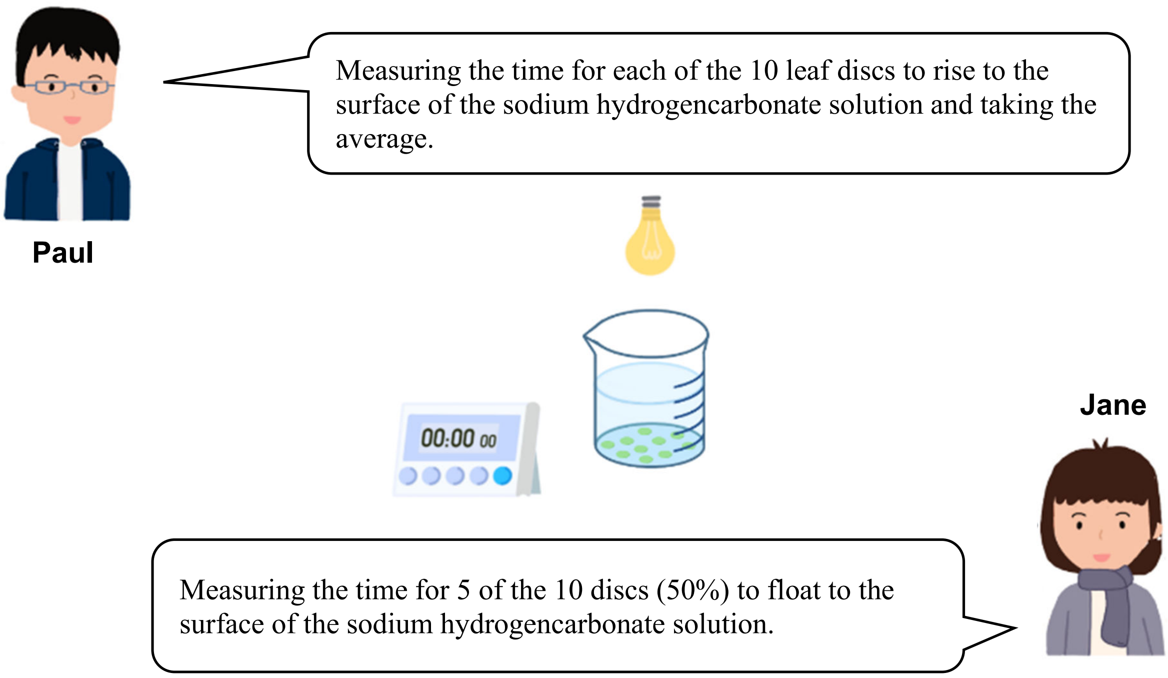
• Vacuumed spinach leaf discs	• 25-mL Measuring cylinder	• 25-mL Beaker
• Table lamp	• 1% Sodium hydrogencarbonate solution	• 1% Sodium hydrogencarbonate solution with herbicides *X*, *Y*, and *Z*
• Timer	• Forceps	

Possible questions

1. Explain whether each of the following modifications can shorten the time of the experiment:

	Yes	No	Your reasons
(a) Use a table lamp with higher light intensity	☐	☐	
(b) Use a larger volume of sodium hydrogencarbonate solution	☐	☐	

2. Your biology teacher advised the class to put 10 leaf discs in each beaker and measure the time for the leaf discs to rise. The following shows the conversation between your classmates about how to measure the time:

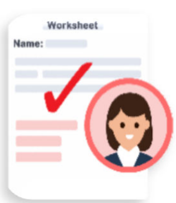


(a) Explain why the method proposed by Paul can enhance the reliability of the results.
(b) Explain why the method proposed by Jane is more accurate than that proposed by Paul.

3. The retardation index (RI) refers to the ratio between the time it takes for half of the leaf discs to float to the surface in a solution containing a herbicide to the time taken by the control (a solution without herbicide).

$$\textbf{RI} = \frac{\text{Time for the leaf discs to float to the surface in a solution with a herbicide}}{\text{Time for the leaf discs to float to the surface in a solution without a herbicide}}$$

Explain how the RI can be used to determine the effect of three herbicides on the photosynthesis of leaf discs.



Notes for teachers

- Q.1 assesses students' understanding of the factors that can affect the rate of photosynthesis of the leaf discs.
- Q.2 assesses students' ability to explain the strategy for averaging the effect of variations within a sample and identify the strengths and limitations of the alternative measurement method of the dependent variable.
- Q.3 assesses students' ability to relate the RI to the dependent variable and apply their biological knowledge about photosynthesis.

The following are examples of students' responses to Q.2(a):

Sample 1

(a) Explain why the method proposed by Paul can enhance the reliability of the results

Paul uses 100% of the data but Jane only uses 50% of the data. The larger the number of sample, the higher the reliability.

Sample 2

(a) Explain why the method proposed by Paul can enhance the reliability of the results

the more the leaf discs being tested, the more data we get. By taking averange, we can eliminate the effect of the extreme cases and increase the reliability of the result.

Sample 3

(a) Explain why the method proposed by Paul can enhance the reliability of the results

It is because Paul's method is repeating the experiment by having 10 leaf disc. However, Jane's method only need, messure the time of the fifth leaf disc float but without repeating.

Sample 4

(a) Explain why the method proposed by Paul can enhance the reliability of the results

Since each leaf discs treated with weedkillers may have some differences in photosynthetic rate By taking average time for leaf discs float with 10 leaf discs, this can minimize the individual differences between leaf discs as some discs float faster while others float later. Thus, reliability can be enhanced.

About the samples

- Sample 1 only addressed the concept of sample size but did not explain how a larger sample size can lead to more reliable data.
- Sample 2 incorrectly stated that the effect of the extreme cases would be eliminated. In fact, the influence of the extreme cases can be averaged out rather than eliminated.
- Sample 3 conflated including more samples within each trial with repeating the entire experiment.
- Sample 4 more clearly explained the variability in photosynthesis rate across different leaf discs, and how taking the average would allow the effect of these individual differences to be averaged out.

The following are examples of students' responses to Q.2(b):

Sample 1

(b) Explain why the method proposed by Jane is more accurate than that of Paul.

Jane's method is more accurate as it ~~take~~ measure time for the 5 ~~dis~~ leaf discs float on the surface first. The results time is more accurate and with less difference between the time of first 5 leaf discs to float, getting a more accurate result as there are less individual differences

Sample 2

(b) Explain why the method proposed by Jane is more accurate than that of Paul.

As the method of Jane excluded the extreme cases, such as the leaf discs which floats in a very short or long time, and use the median of the result, therefore it is more accurate.

Sample 3

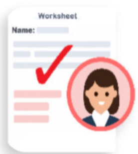
(b) Explain why the method proposed by Jane is more accurate than that of Paul.

There might be leaf discs that take an extra long/short time to rise up, making extreme data. Hence, this method can eliminate the extreme data that makes the result inaccurate and the results can be closer to the true value, but Paul's method includes the extreme data in calculations so the results obtained may deviate greatly from the true value, so Jane's method is more accurate.

About the samples

- Sample 1 did not clearly explain how using data with less variability can result in more accurate data.
- Sample 2 incorrectly stated that the method can eliminate the extreme cases. Indeed, this method does not disregard the extreme data points as the time for the first five leaf discs to rise to the surface of the solution is still measured.
- Sample 3 correctly noted that eliminating extreme data points can lead to results that are closer to the true underlying value, by reducing the distorting effect of outliers.

Laboratory Manual



Notes for teachers

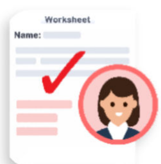
- Teachers can distribute the manual for students to read and prepare before the investigation.
- Teachers can ask questions to check if students fully understand the procedures (e.g., how would you position the table lamp and the beakers? Why?).
- The *Supplementary Resource* section contains the list of materials.
- Scan the QR code to view the process of the experiment.

Task 3

- Read the following procedures to carry out the investigation.

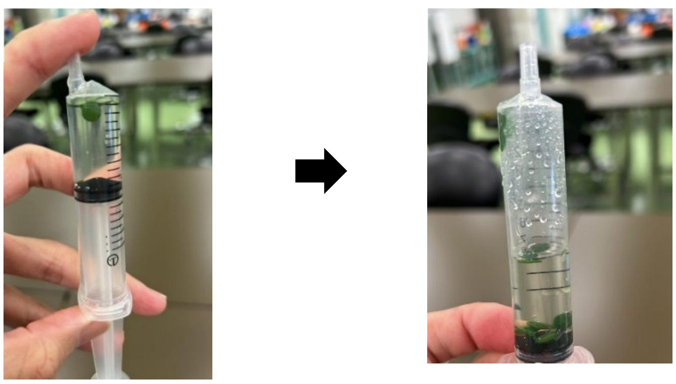
Procedure

1. Cut at least 40 leaf discs from the green leaf of the spinach plant (*Spinacia oleracea*) with a hole punch. (Avoid the main leaf veins.)
2. Place the cut leaf discs on moist tissue paper to prevent them from drying out during preparation.
3. Pour 15 mL of 1% sodium hydrogencarbonate solution into a plastic cup.
4. Place at least 10 leaf discs in the plastic cup.
5. Repeat *Steps 3* and *4* using 1% sodium hydrogencarbonate with *Herbicides X*, *Y*, and *Z*, respectively.
6. Vacuum all the leaf discs for 1 minute.
7. Repeat *Step 6* 1 more time.
8. Use forceps to transfer 10 leaf discs into each 25-mL beaker containing 15 mL of the test solution and control.
9. Place all beakers under the table lamp provided. Make sure that all leaf discs receive even light intensity and heat from the table lamp.
10. Switch on the lamp, and immediately start the timer.
11. Record the time taken for 50% (i.e., 5 discs) of the leaf discs in each of the beakers to float to the surface of the solution (i.e., ET50).
12. Obtain data from two other groups.

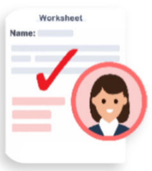


Notes for teachers

- The vacuuming step can be done by using a syringe.



Teacher Notes 2



Notes for teachers

- The following are possible questions that teachers can use to guide students in thinking about or assessing their scientific inquiry skills related to data analysis and interpretation.
- Some student work samples are shown to illustrate possible student thinking to some questions.

<u>Task 4</u>

A checklist (see p.13) can help students understand the requirements for creating a table.

Possible questions

1. Present your group and your classmates' results using a table.
   (Make sure that your table includes the retardation index and a title.)

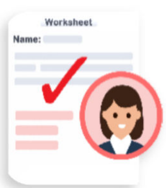
2. Which of the following claims about the mode of action can be made?
   (Put a '✓' into the appropriate box(es). You can choose *one* or *more* answers.)

A.	Herbicide *X* is a photosynthesis inhibitor.	☐
B.	Herbicide *Y* is not a photosynthesis inhibitor but a cell division inhibitor.	☐
C.	Herbicide *X* can inhibit photosynthesis more than herbicides *Y* and *Z*.	☐
D.	Both Herbicides *Y* and *Z* are plant growth regulators.	☐

3. (a) Which of the following ways did your group use when pooling class data:
   (1) Selecting groups with data similar to your group
   (2) Selecting groups randomly

   Explain your reasons for why this method is appropriate:

   (b) Explain why obtaining data from other groups can reduce the impact of measurement errors.



Notes for teachers

- Q.1 assesses students' ability to record data properly and to construct a result table that can clearly document the data.
- Q.2 assesses students' ability to make claims based on the data available. The method used in this investigation can only identify whether it is a photosynthesis inhibitor but not other modes of action.
- Q.3 and Q.4 assesses students' understanding of how to pool class data to reduce the impact of errors and the explanation.

The following are some examples of students' responses to Q.1:

Sample 1

1. Present your group and your classmates' results using a table:
   (Make sure that your table include the retardation index and a title)

Time taken for leaf discs to float to the surface of sodium hydrogen carbonate with different Herbicide

	Herbicide X	Herbicide Y	Herbicide Z	without Herbicide
Group 6	infinity	26min	13min 39s	infinity
Group 2	infinity	9min 2s	23min 18s	14min 27s
Group 1	infinity	9min	48min 7s	11min 49s
RI	infinity	1.4	1.92	1

Sample 2

1a) Present your group and your classmates' results using a table:
(Make sure that your table include the retardation index and a title)

	Time taken for solution X	RI of X	Time Taken for Solution Y	RI of Y	Time taken for solution Z	RI of Z	Time taken for control
Group 3	∞	∞	20min 49s = 1249s	≈ 1.96	4min 3s = 243s	≈ 0.38	10min 36s = 636s
Group 6	∞	∞	13min 23s = 803s	≈ 0.60	22min 40s = 1360s	≈ 1.00	22min 35s = 1355s
Group 2	∞	∞	19min 51s = 1191s	≈ 0.76	32min 53s = 1973s	≈ 1.25	26min 14s = 1574s

Effects of different herbicides acting with leaf ~~disc~~ discs on photosynthesis

Sample 3

(1a) Present your group and your classmates' results using a table:

(Make sure that your table include the retardation index and a title)

The time taken for the leaf discs to float in different herbicide

Type of herbicide	Group's		Collen's gp		Wendy's gp	
	time (s)	Retardation index	time (s)	Retardation index	time (s)	Retardation index
control	1670	1	1205	1	18[illegible]	1
X	>2009	>1.202	>2000	>1.66	X	X
Y	1702	1.02	672	0.56	273	0.15
Z	312	0.19	1800	1.49	1908	1.03

About the samples

- These samples show varying sophistication in terms of their ability to construct a proper table.
- Common mistakes include the absence of titles, lack of units in the headings of the table columns/rows, and inclusion of calculation within the table.
- The following shows a checklist that may be provided to students to guide them to construct a table.

Checklist on how to construct a table

1. Use a ruler to construct a table that presents the raw data. ☐
2. Place the independent variable in the first column and the dependent variable in the subsequent columns. ☐
3. Do *not* include calculations in the table. ☐
4. For each column, include a heading with the appropriate unit in brackets (e.g., enzyme activity [1/min]). ☐
5. Do *not* include units within the body of the table, only in the column headings. ☐
6. Record the raw data to a number of decimal places appropriate to the resolution of the equipment. ☐
7. Record all the raw data of the same type to the same number of decimal places. ☐
8. Record processed data up to one significant figure more than the raw data. ☐
9. Include a title for the table. ☐

The following are some examples of students' responses to Q.3(a):

Sample 1

3. What of the following ways did your group use when pooling class data:
   A. Selecting groups with data similar to your group
   B. Selecting groups randomly

Answer: B

Explain your reasons

Collecting data from some random groups can increase the reliability. It ensures all data, including some extreme data will be counted.

Sample 2

3. What of the following ways did your group use when pooling class data:
   A. Selecting groups with data similar to your group
   B. Selecting groups randomly

Answer: B

Explain your reasons

Selecting groups randomly can prevent selection bias and the result is not affected by human factor.

Sample 3

3. What of the following ways did your group use when pooling class data:
   A. Selecting groups with data similar to your group
   B. Selecting groups randomly

Answer: B

Explain your reasons

It can increase validity. If the data of your own group is inaccurate, selecting groups with data similar to your own group will also inaccurate. The result will be inaccurate after taking average. As a result, the data ~~should~~ from other groups should be selected randomly for reducing the chance of obtaining inaccurate data.

About the samples

- All the samples chose the correct strategy to pool class data. However, they differed in the sophistication of their reasoning.
- Some samples included invalid reasons, such as including extreme data and removing the errors (i.e., not affected by human factors) (rather than reducing the impact of errors), as seen in Samples 1 and 2.

Supplementary Resources

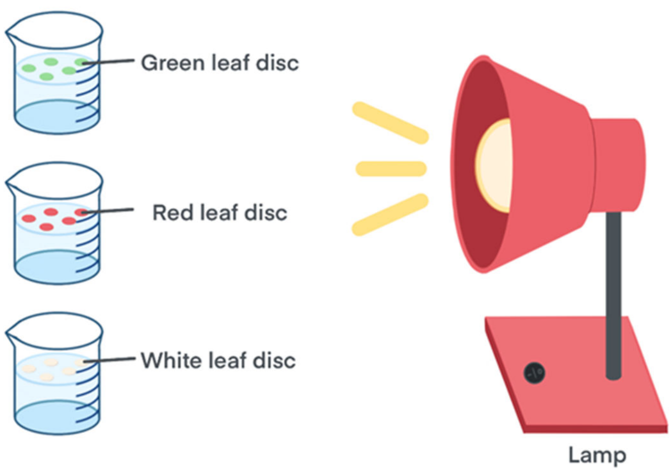
Possible Modifications

1. **Sinking of floating leaf discs in darkness**
   - Teachers may ask students what would happen to the floating leaf discs if they are kept in darkness for 30 minutes and ask them to provide the reasons.
   - See Steucek & Hill (1985) for more information.

2. **Investigating the effect of light colour on the photosynthesis of the leaf discs**
   - An LED (RGB light bulb) may be used to investigate the effect of light colour on the photosynthesis of the leaf discs. See also the *Cat Grass Investigation*.
   - Note that even though the effects of light colour on photosynthesis are not within the scope of the curriculum, teachers can still ask students to investigate these effects. The focus should be on how students use their data to construct claims about the effects based on evidence from their data rather than on the theory behind the effects.

Scan the QR code for more information.

3. **Investigating the photosynthesis of different leaf colours.**
   - The set-ups can also be used to investigate the photosynthesis of leaves with different colours.



Technician Notes

Materials for Task 4

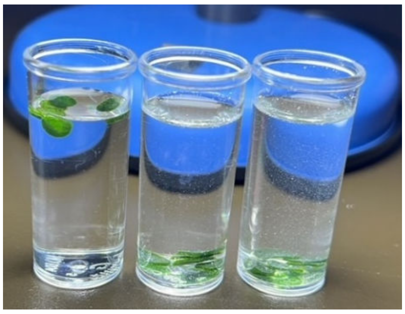
Chemicals to be prepared

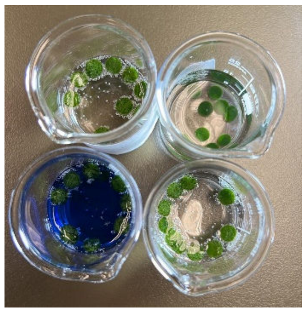
- 1% sodium hydrogencabonate solution (1 L) (Dissolve 10 g sodium hydrogencabonate in 1 L distilled water.)
- Herbicide *X* (0.005% Atrazine)
  - Atrazine stock solution 1 mg/mL = 0.05 g in 50 mL DMSO
  - Add 50 mL of Atrazine (1 mg/mL) stock solution and 10 g of sodium hydroegncarbonate, then make up the volume to 1 L.
- Herbicide *Y* (0.005% 2,4–D)
  - 2,4–D stock solution 1 mg/mL = 0.05 g in 50 mL DMSO
  - Add 50 mL of 2,4–D (1 mg/mL) stock solution and 10 g of sodium hydroegncarbonate, then make up the volume to 1 L.
- Herbicide *Z* (0.001% DCPIP)
  - Add 10 mL of 0.1% DCPIP (0.1 g in 100 mL distilled water), 10 g sodium hydrogencabonate solution, make up to 1 L.

Materials for each group

• Hole punch	• Forceps	• Pen
• Spinach leaves	• Petri dish	• 1% sodium hydrogencarbonate
• Plastic cup X 4	• Cotton wool (moist)	• 1% sodium hydrogencarbonate with Herbicide *X*
• Vacuum pump	• 25 mL beaker X 4	• 1% sodium hydrogencarbonate with Herbicide *Y*
• Vacuum chamber	• Timer	• 1% sodium hydrogencarbonate with Herbicide *Z*

Note: Select fresh spinach leaves.





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