NAME: _____ CLASS: _____ INDEX: _____

CATHOLIC JUNIOR COLLEGE
JC2 PRELIMINARY EXAMINATIONS
Higher 2

MARKING SCHEME

BIOLOGY
Paper 4 Practical

9744 / 04
25 August 2023
2 hours 30 min

Candidates answer on the Question Paper
Additional Materials:

READ THESE INSTRUCTIONS FIRST

Write your index number and name on all the work you hand in.
Give details of the practical shift and laboratory, where appropriate, in the boxes provided.

Write in dark blue or black pen.
You may use a HB pencil for any diagrams or graphs.

Do not use staples, paper clips, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate. You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.
The number of marks is given in brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
TOTAL	

This document consists of **XX** printed pages and **X** blank page.

[Turn over

Answer **all** questions.

1 During photosynthesis in leaves, carbon dioxide enters through stomata and diffuses through intercellular air spaces to the mesophyll.

Leaf discs submerged in solution can be used to investigate photosynthesis.

To investigate the effect of carbon dioxide concentration on the rate of photosynthesis in leaf discs, you will:

- prepare different concentrations of sodium hydrogencarbonate solution, as a source of dissolved carbon dioxide.
- measure the rate of photosynthesis by recording the time taken for submerged leaf discs to reach the surface due to the build-up of oxygen in the air spaces.

You are provided with:

- 30 cm^3 of 10.0 g dm^{-3} sodium hydrogencarbonate solution, labelled **H1**
- distilled water, labelled **W**
- fresh, green leaves from plant species **A**, in a Petri dish labelled **A**

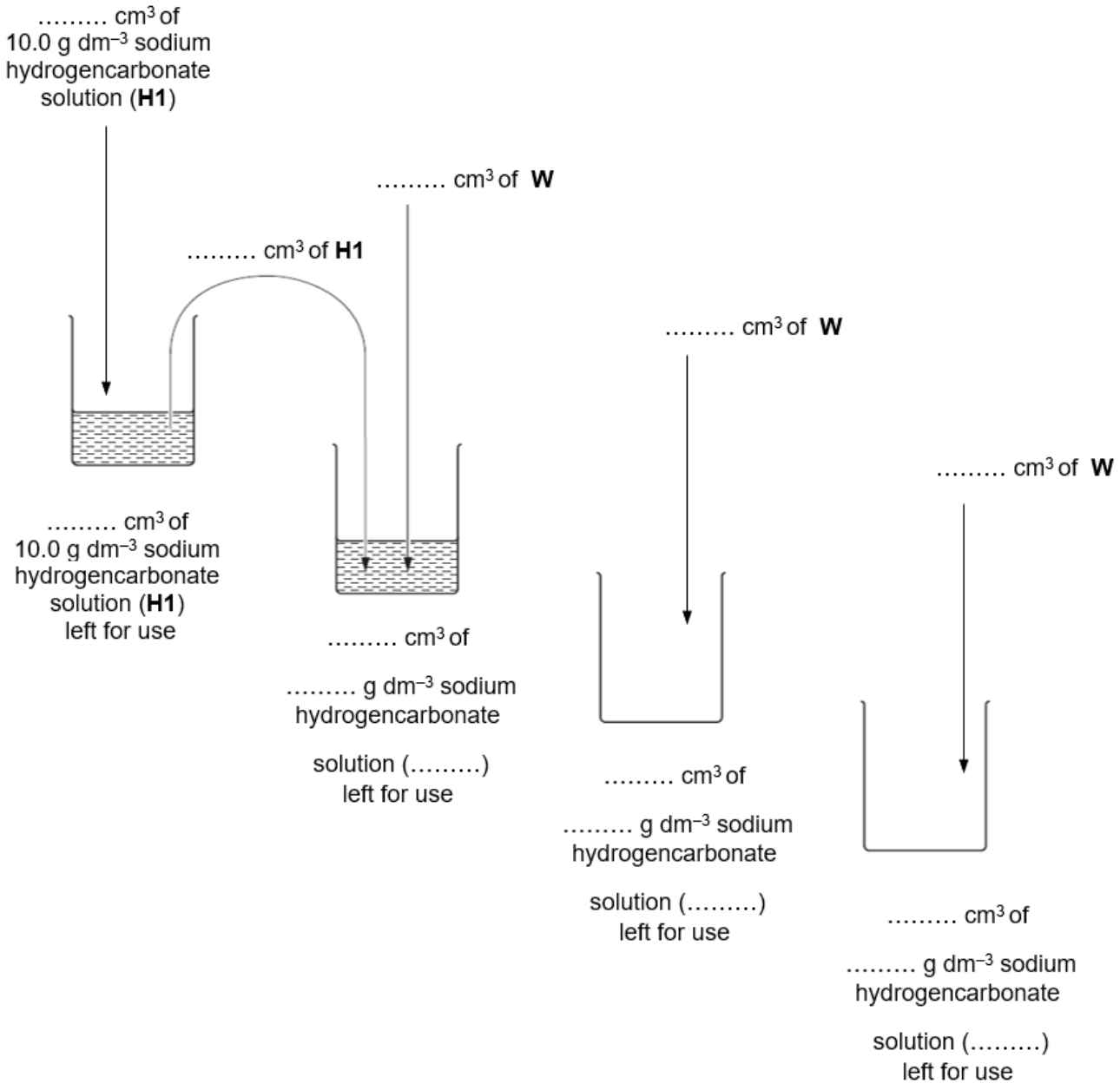
(a) Carry out a serial dilution of the 10.0 g dm^{-3} sodium hydrogencarbonate solution, **H1**, to reduce the concentration of the sodium hydrogencarbonate solution by **half** between each of three successive dilutions, to give **H2**, **H3** and **H4**.

You are required to make up a sufficient volume of each concentration of sodium hydrogencarbonate solution in the specimen tubes provided so that, once the serial dilution has been completed, there is at least a volume of 10 cm^3 for each solution.

(i) Complete Fig. 1.1 on page 3 to show how you will carry out your serial dilution.

For each specimen tube,

- draw one curved arrow with a label, above the specimen tube, to show the **volume** and **concentration** of hydrogencarbonate solution added from the previous tube.
- state the **volume** of **W** added to prepare the concentration.
- state, under the specimen tube, the **volume** and **concentration** of hydrogencarbonate solution left for use in the investigation.


..... cm³ of
10.0 g dm⁻³ sodium
hydrogencarbonate
solution (H1)
..... cm³ of W
..... cm³ of H1
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10.0 g dm⁻³ sodium
hydrogencarbonate
solution (H1)
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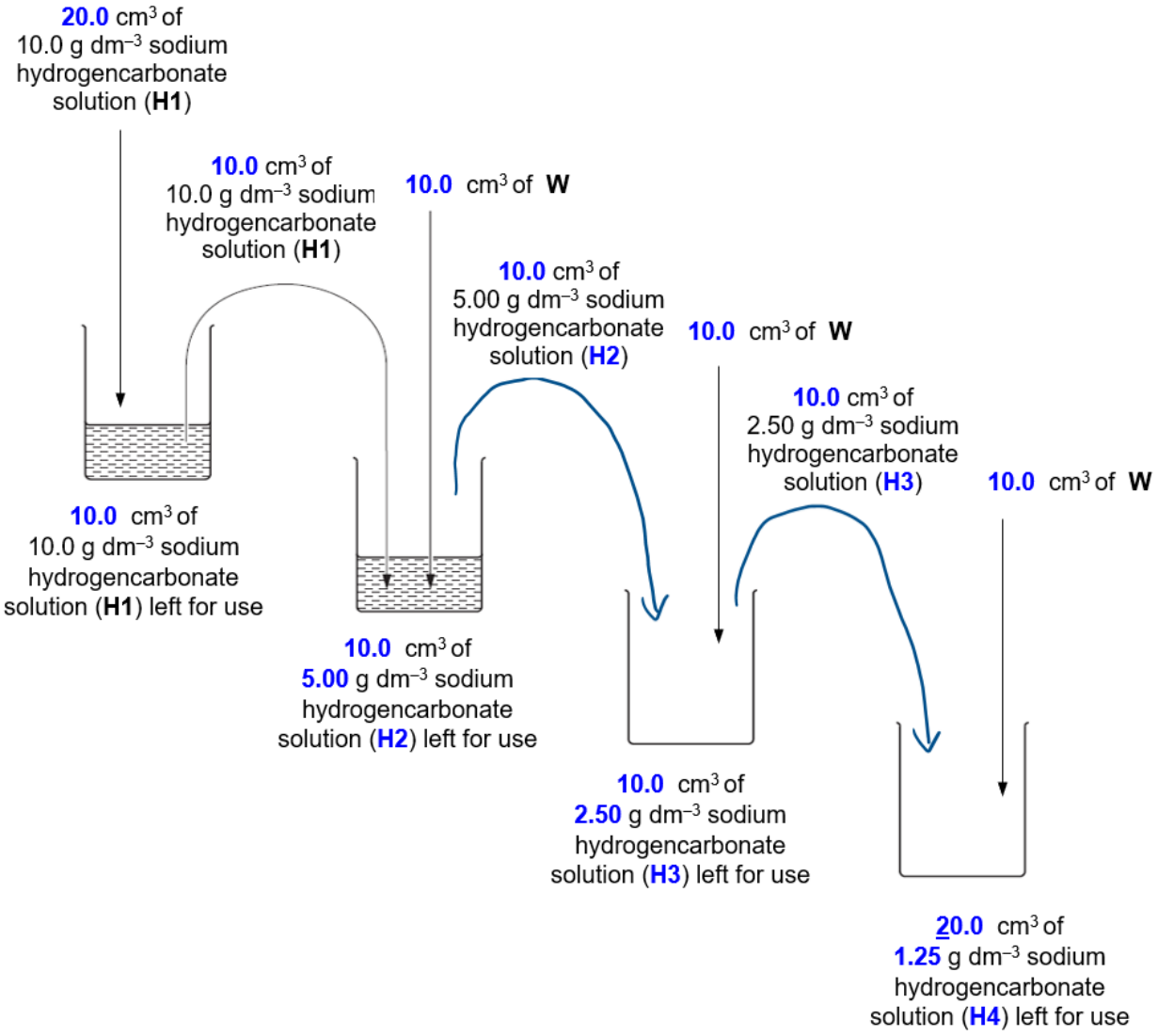
Fig. 1.1

[3]



Mark Allocation

[C1] Correct final concentrations of **5.00, 2.50, 1.25** [Ignore d.p] with correct labelling of **H1, H2, H3** and **H4** at the bottom of each specimen tube

[C2] Correct **volumes** and **concentration** to make up at least 10.0 cm^3 of H2, H3 and H4 (after all dilutions completed) and remaining volume left to use for all solutions are ≥ 10 cm^3)
[Note: for H4, volume remaining is either 20.0 cm^3 (if there is not further removal) or 10.0 cm^3 if there is volume removed]

[P] All volumes to be listed / given to **1 d.p.** and concentrations given to **3 s.f.**

*Read steps **1–14** and prepare the table in **(a)(iii)** before proceeding.*

1 Using a plastic drinking straw, cut out a leaf disc by placing an open end of the straw on a leaf from plant species **A**, while supporting the leaf from behind with your finger. Push the straw through the leaf on to your finger to cut out a disc. The disc will stick onto the end of the straw. Repeat until you have 12 leaf discs of uniform size.

2 Place the discs in a Petri dish containing distilled water, **W**. You may need to tap the straw a few times to detach the discs.

3 Remove the plunger of a 10 cm^3 syringe.

4 Place all the leaf discs in the barrel of the syringe, using forceps. Make sure that the leaf discs are at the bottom of the syringe barrel. Replace the plunger of the syringe and push it down until it almost touches the leaf discs.

5 Draw up 5 cm^3 of distilled water, **W**, from the Petri dish into the syringe.

6 Turn the syringe so that the nozzle is pointing upwards and tap the sides so that air bubbles rise to the top. Push the plunger in until the nozzle of the syringe is filled with water.

7 Place a finger tightly over the nozzle of the syringe to form an airtight seal. Firmly draw out the plunger as far as you can, as shown in Fig. 1.2, and then gently allow the plunger to return to its starting position.

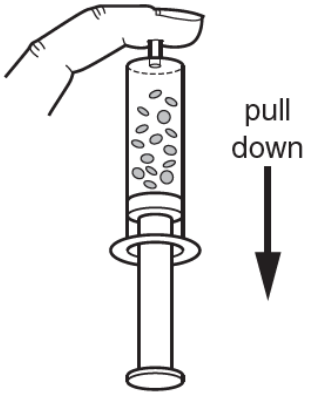


Fig. 1.2

8 Remove your finger from the nozzle and shake the syringe gently or tap the side to remove gas bubbles. The leaf discs should sink.

9 If discs are still floating on the surface, repeat steps **6-8** until nearly all leaf discs have sunk. You may have to repeat steps **6-8** several times.

(ii) State the purpose of steps **6** to **8**.

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[1]

To **remove any gases** from the air spaces in the leaf discs (so that they will sink in sodium hydrogencarbonate solution)

[Reject: ref. to gases on the outside / surrounding]

10 Carefully pull the plunger out of the syringe and pour the water and leaf discs back into the Petri dish. If any leaf discs remain in the syringe, use the glass rod to remove them.

11 Put one leaf disc into each of the specimen tubes containing the same volume of sodium hydrogencarbonate solutions. Swirl each tube and use a glass rod to push the leaf disc to the bottom. If any leaf disc floats it should be removed and replaced with a fresh leaf disc that sinks.

12 When all specimen tubes contain a leaf disc that has sunk to the bottom, place the specimen tubes 10 cm from a bench lamp.

13 Switch on the bench lamp and start a stopwatch.

14 Record the time taken for each leaf disc to reach the surface, in **(a)(iii)**.
If any leaf discs have not reached the surface after 15 minutes, record 'more than 900'.

(iii) Record your results in a suitable table in the space below.

Concentration of sodium hydrogen carbonate solutions / g dm^{-3}	Time taken for leaf disc to reach the surface / s
10.0	Shortest
5.00	
2.50	
1.25	Longest

[H] – ruled table drawn, with Independent Variable on the leftmost column and with appropriate & correct column headings and units for both independent and dependent variables:
- Concentration of sodium hydrogen carbonate solutions / gdm^{-3}
- Time taken for leaf disc to reach the surface / s

[Reject: short forms used for concentration; 'sec' instead of 's' for seconds]

[O] – Record all 4 **concentrations** to 3 sig. fig. and **time** as whole number; no units in body of table

[T] – Correct Trend (records shortest time for the highest concentration of sodium hydrogencarbonate solution (H1), increasing time as concentration of sodium hydrogencarbonate decreases to minimum (H4)

[Reject: if all time readings is 'more than 900']

[3]

(iv) Describe a suitable control that could have been used for this investigation.

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[1]

Control is to replace hydrogencarbonate solution with

EQUAL AMOUNT (or state the volume used) of **distilled water** / W

[Reject 0% hydrogen carbonate solution;

1 leaf disc and

place specimen tube 10 cm from light source

and

either repeat steps 11-14 or subject to same experiment steps

(v) The validity of your results can be increased by performing replicates.

State and explain **one other** improvement to this procedure that could improve the validity of your results.

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[2]

1a. *Improvement*: Perform / run / carry out **separate experiments for each concentration** of sodium hydrogencarbonate solution / i.e. place one tube in front of the lamp each time

1b. *Ratioale*: So that **light intensity / amount of light** received by the leaf disks is the **same/ equal**
[Idea of: since doing the experiments at the same time will cause the leaf disk to have different position relative to the lamp or light source/ some tubes are at an angle to lamp, some are directly in front of the lamp

Or

2a. Improvement: Use a heat filter / shield / e.g a cup of water placed near the lamp to absorb heat /use a cool light source e.g. LED lamp which emits less heat

2b. *Rationale*: To ensure a more constant temperature in the specimen tubes / experimental set-up / since heat will cause an increase in temperature + link to **enzymatic** reactions

Or

3a. *Improvement:* Use more leaf discs made from the same leaf / plant (i.e. to increase sample size); get an average of time taken to reach surface

3b. *Rational*: To increase sample size to spread out or reduce variability in number chloroplasts present / number of airspaces (in the leaf disc)

AVP: To ensure same physiological state of cells (from the same leaf)

(vi) A student used this method to investigate photosynthesis in a different species of plant, **B**. She found that the higher the concentration of sodium hydrogencarbonate, the faster the leaf discs reached the surface.

Explain this relationship.

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[3]

1. An increase in concentration of sodium hydrogencarbonate (which represents/causes an increase in dissolved carbon dioxide concentration / availability) increases the rate of the **light independent reactions / Calvin cycle** / carbon fixation

2. This uses up **NADPH** and **ATP** from **light dependent reactions** / leads to faster production of NAD and ADP by light dependent reactions

3. (faster rate of light dependent reactions) leads to faster **photolysis of water** to produce **oxygen** and hence the faster the leaf discs reach the surface;

(b) Global warming and associated precipitation changes will negatively impact on many agricultural ecosystems. Major food production areas are expected to experience reduced water availability and increased frequency of drought over the coming decades.

Almost all water used for plant growth is lost to the atmosphere by transpiration through stomatal pores on the leaf epidermis.

Several approaches to improve drought tolerance and water-use efficiency through the modification of stomatal traits have been tested in the model plant *Arabidopsis thaliana*. The *SDD1* gene is involved in the regulation of stomatal density and distribution in *Arabidopsis thaliana*. A loss of function mutation in *SDD1* gene was shown to increase stomatal density in mutant plants in a previous study.

A student investigated the stomatal density in two lineages of plants, **A** and **B**, to find out if overexpression of *SDD1* gene can decrease stomatal density. Plants in lineage A overexpressed *SDD1* gene, while plants in lineage B expressed *SDD1* gene at normal levels.

Table 2.1 shows the results of the investigation.

Table 2.1

sample number	stomatal density / mm^{-2}	
	lineage A	lineage B
1	181	190
2	182	191
3	190	192
4	178	193
5	184	194
6	183	194
7	181	196
8	179	197
9	190	190
10	179	192
mean ($\bar{x}$)	182.7	193.1
standard deviation (s)	4.27	2.81
variance (s^2)	18.23	7.89

(i) Complete Table 2.1 by calculating the variance (s^2) for the stomatal density of two lineages: lineage **A** and lineage **B**. [1]

(ii) A *t*-test can be used to determine whether there is any significant difference between the stomatal density in lineage **A** and lineage **B**.

Calculate the value of *t* and the number of degrees of freedom, using these formulae:

$$t = \frac{|\ \bar{x}_1 - \bar{x}_2\ |}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}} \qquad v = n_1 + n_2 - 2$$

key to symbols

s = standard deviation
$\bar{x}$ = mean
n = sample size (number of observations)
v = degrees of freedom

Show your working.

$$t = \frac{|\ 182.7 - 193.1\ |}{\sqrt{\left(\frac{18.23}{10} + \frac{7.89}{10}\right)}} = 6.43$$

value of *t* = 6.43

number of degrees of freedom = 18

[2]

Mark Allocation
[1] for working (substitution of correct values into formulae)
[1] correct calculation / correct value of t and degrees of freedom

(iii) State the null hypothesis.

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[1]

There is no **significant** difference between the (means of) stomatal density in lineage A and lineage B;

Table 2.2 shows the critical values for t at different probabilities and degrees of freedom for one-tailed test and two-tailed test.

Table 2.2

degrees of freedom	probability, *p*, for one-tailed test			
	0.25	0.05	0.025	0.005
	probability, *p*, for two-tailed test			
	0.5	0.1	0.05	0.01
1	1.00	6.31	12.71	63.66
2	0.82	2.92	4.30	9.92
3	0.76	2.35	3.18	5.84
4	0.74	2.13	2.78	4.60
5	0.73	2.02	2.57	4.03
6	0.72	1.94	2.45	3.71
7	0.71	1.89	2.36	3.50
8	0.71	1.86	2.31	3.36
9	0.70	1.83	2.26	3.25
10	0.70	1.81	2.23	3.17
11	0.70	1.80	2.20	3.11
12	0.70	1.78	2.18	3.05
13	0.69	1.77	2.16	3.01
14	0.69	1.76	2.14	2.98
15	0.69	1.75	2.13	2.95
16	0.69	1.75	2.12	2.92
17	0.69	1.74	2.11	2.90
18	0.69	1.73	2.10	2.88
19	0.69	1.73	2.09	2.86
20	0.69	1.72	2.09	2.85

(iv) Use Table 2.2 and your answers to **(b)(ii)** to decide whether the null hypothesis should be accepted or rejected.

Explain your answer.

accept or reject null hypothesis Reject null hypothesis

explanation For v = 18, p = 0.05, $t_{calculated}$ of **3.34** is **higher** than $t_{critical}$ of 2.10.

Conclusion: There is a significant difference between the (mean) stomatal density of lineage A and lineage B

[Reject: no critical t-value stated; critical t-value from one-tailed test]

[2]

[Total: 19]

2 Bromelain is a proteolytic enzyme that catalyses the hydrolysis of proteins into their amino acid building blocks. This enzyme can be found in fruits such as pineapple.

Gelatin contains structural proteins derived from animal tissues high in collagen. It sets into a gel-like structure upon cooling after dissolving in warm water. The action of bromelain on gelatin gel results in liquification of the gel.

(a) Design an experiment to investigate the effect of different concentrations of bromelain on gelatin breakdown.

In your plan, you must use:

- gelatin gel that has been prepared by dissolving 54 g of gelatin powder in 100 cm^3 of hot water and the mixture transferred into 5 separate large specimen tubes and allowed to set in an ice water bath for 15 minutes
- a piece of pineapple
- mortar and pestle
- tea filter bag
- small specimen tubes
- syringes

Your plan method should:

- have a clear and helpful structure so that the method described could be repeated by anyone reading it
- include details to ensure that results are accurate and repeatable as possible
- identify the dependent and independent variables
- use the correct technical and scientific terms
- only make use of the apparatus and materials provided

[6]

Mark Scheme

Point		Guidance
A.	Variable	Independent variable: different concentration of bromelain / pineapple juice Dependent variable: height of gelatin / volume of liquid collected in a given time.
B.	accurate and repeatable	Perform 2 more replicates AND calculate mean or identify/eliminate/remove anomalies
C.	Must use	Students used all the "must use" apparatus
D.	Dilution table	Show dilution table, using serial dilution. **Reject: simple dilution**
E.	Method to correctly collect dependent variable	
F.	Method to prepare juice extract	
G.	Reference to suitable control	
H.	Standardise gel	Volume / height of gelatin

Suggested Procedure

1. Crush the whole slice of pineapple into pulp using pestle and mortar
2. Add in 5 cm^3 of water and crush for another 1 minute
3. Pour the mixture through the tea filter bag and extract the pineapple juice
4. Collect the filtrate in a beaker.
5. Perform serial dilution based on the table below.

Concentration of bromelain solution / %	Volume of solution from previous concentration / cm^3	Volume of DI water / cm^3	Total volume / cm^3
100.0	5.0	0.0	5.0
50.0	5.0	5.0	5.0
25.0	5.0	5.0	5.0
12.5	5.0	5.0	5.0
6.25	5.0	5.0	5.0

6. Once the gelatin set, label the different specimen tube with the different concentration of bromelain solution used using a marker.
7. Using the specimen tube for 100% bromelain solution, add in 5 cm^3 of bromelain solution and start the stopwatch immediately.
8. At the end of 10 minutes, pour the liquified gelatin with the bromelain solution into a specimen tube and measure the volume collected using the 5ml and 1ml syringes.

9. Record the volume collected in the table below.

Concentration of bromelain solution / %	Volume of liquid collected / cm^3
100.0	5.60
50.0	5.25
25.0	5.10
12.5	5.00
6.25	5.00

10. Repeat step 10 to 11 for the rest of the bromelain solution.

(i) State and explain one variable that is kept constant in this investigation.

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[2]

1. State: Concentration of gelatin mixture used / mass of gelatin powder
2. Explain: Affects the rate of hydrolysis by bromelain

OR

3. State: Same (source of) pineapple used
4. Explain: different pineapple may have different concentration of enzymes

(ii) State one possible source of error in your investigation and suggest how this can be improved.

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[2]

1. State: Temperature of set up
2. Suggest: use of thermostatically controlled water bath to control for temperature during the reaction

(iii) Suggest a suitable control experiment to show that bromelain is the cause of liquification of gelatin.

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[1]

Control experiment to use boiled and cooled bromelain solution of the same volume, in place of bromelain solution.
(Further qualification not required as it is explained in the question stem).

(iv) Rennin is a protease. However, it does not cause gelatin to liquefy. Suggest and explain why rennin does not breakdown gelatin.

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[2]

1. Idea that the active site of the enzyme rennin does not have a complementary 3D conformation / charge to gelatin
2. Gelatin cannot bind to form enzyme-substrate complex for it to become liquefied.

(b) A student performed a similar experiment at 30^{o}C to investigate the effect of different pH on bromelain activity.

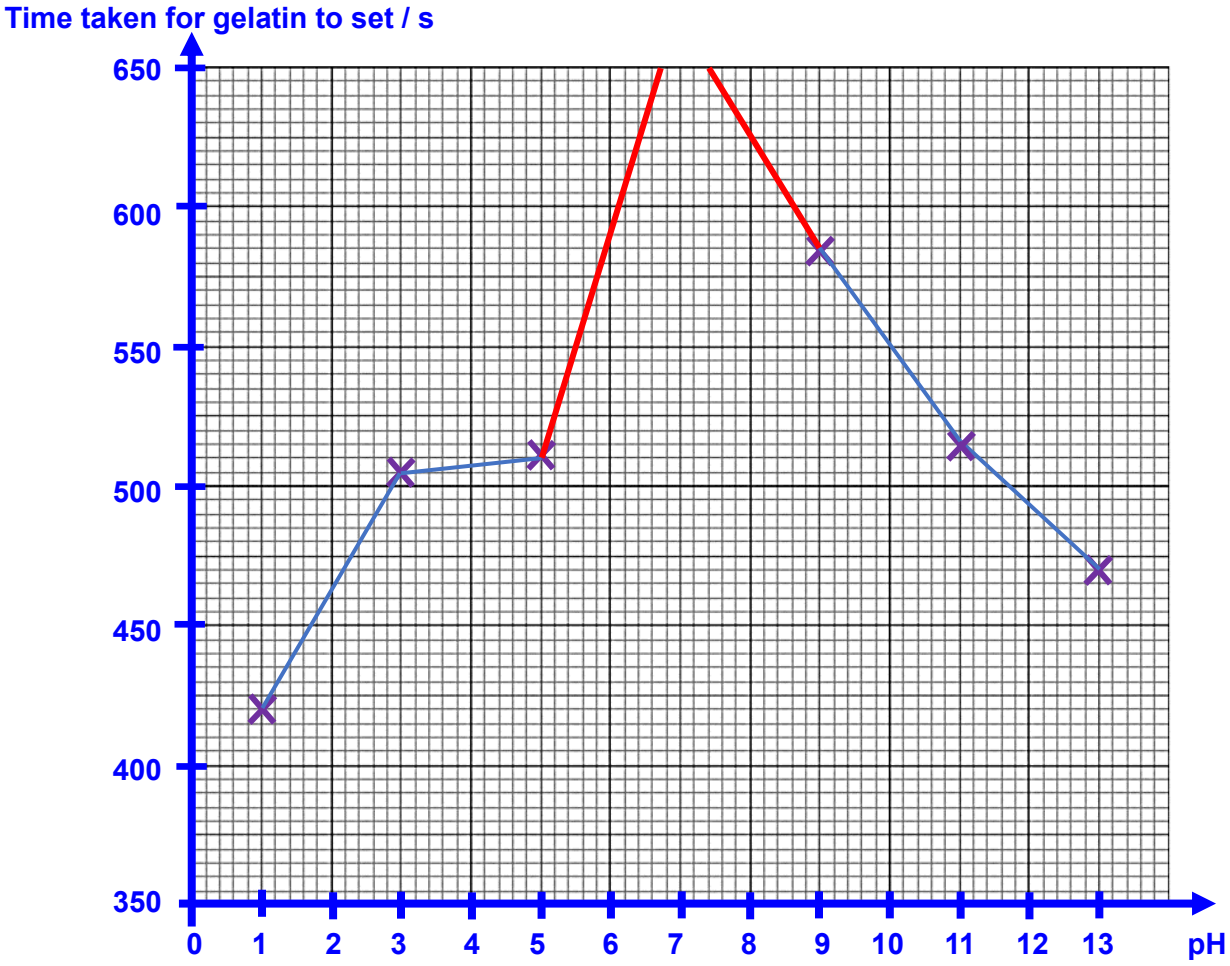
The pineapples were first blended into a juice, and pH buffer added. The time taken for gelatin with the different juice and pH buffer to set were recorded. If the gelatin did not set after 600 seconds, the time recorded will be 'more than 600'.

The results are shown in Table 1.1.

Table 1.1

pH of solution	time taken for gelatin to set / s
1	420
3	505
5	510
7	more than 600
9	585
11	515
13	470

(i) Plot a graph of your results from Table 1.1 in the grid below.



[4]

[S] – Appropriate scale that covers at least ¾ of the grid;

[A] – Correct axes headings with appropriate units
(X-axis – pH of solution; Y-axis – time taken for gelatin to set / s);

[P] – All points correctly plotted; [Note: No pH7 plot point]

[L] – Clean, straight lines drawn, joining point-to-point of existing plot points;
[Accept 2 non-continuous lines drawn or extrapolation (in red)]

(ii) With reference to Table 1.1, explain the effect of pH on the rate of bromelain activity.

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[3]

1. As pH increases from 1 to 7, time taken for gelatin to set increases from 420s to more than 600 s and time taken for gelatin to set decreased to 470 s at pH 13
2. At **pH 7**, bromelain **continuously hydrolyze the bonds of collagen** in gelatin, unable to set hence time taken to set more than 600 s
3. **Below and above pH 7**, change in pH **altered the charges of the acidic and basic R-groups** of amino acids of the enzyme, **disruptiing ionic bonds and hydrogen bonds** that are involved in maintaining the specific three-dimensional structure of the enzyme → bromelain denatured / non-functional

(iii) Suggest why the enzyme bromelain does not digest the proteins in the stomach when fresh pineapple is eaten.

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[1]

pH of stomach is around 3, bromelain will be denatured due to the low pH

[Total: 21]

3 During this question, you will need access to a microscope and slide **S1**.

S1 is a slide of a stained transverse section through a plant leaf.

You are not expected to be familiar with this specimen.

(a) Use the microscope to observe the different tissues in the leaf on **S1**.

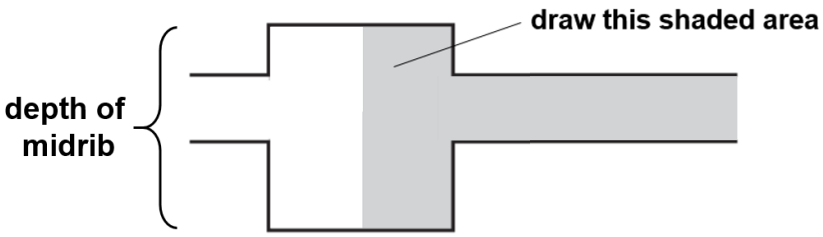
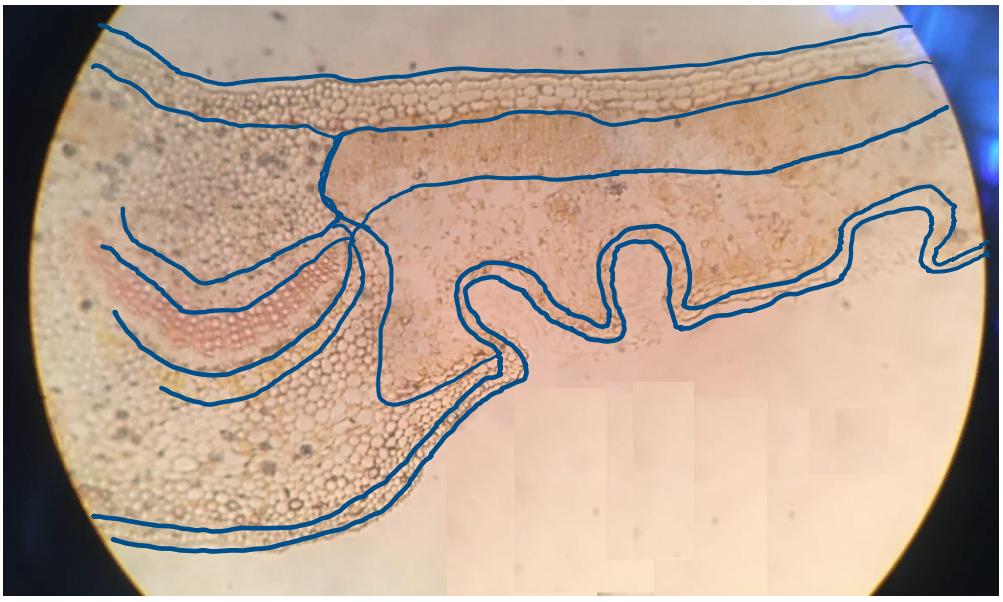


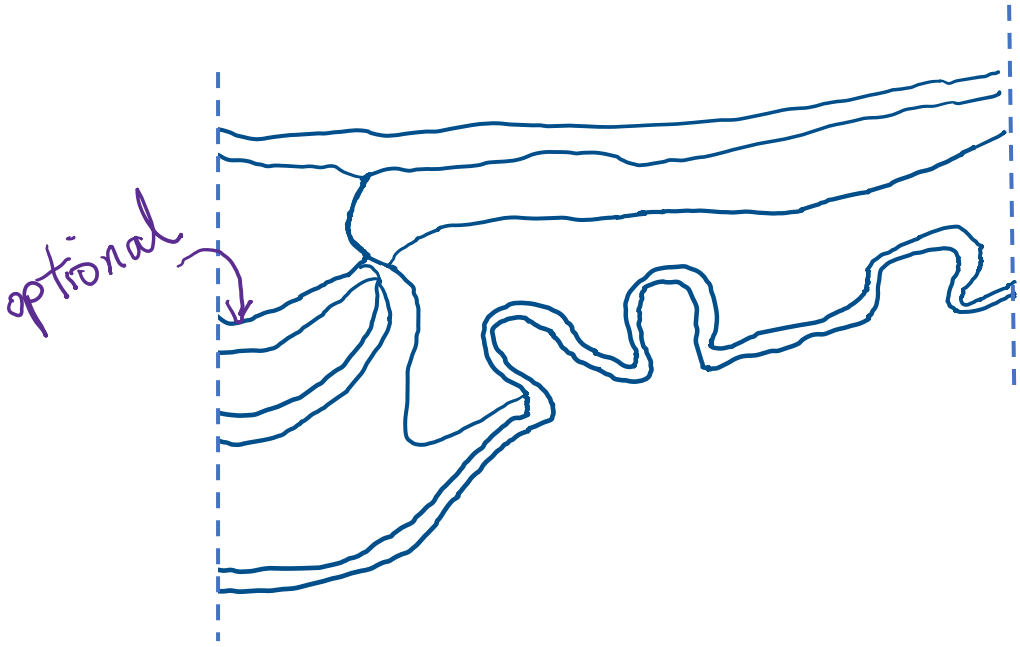
Fig. 3.1

(i) Draw a large plan diagram of the shaded area of the leaf on **S1** shown in Fig. 3.1.

A plan drawing shows the arrangement of different tissues. Your drawing should show the correct shapes and proportions of the different tissues.

No cells should be drawn.





[4]

Marks Allocation:

[S1] - Correct outline (shaded area) drawn; size of drawing at least ¾ of space given;

[S2] - Lines continuous, clear and smooth; No cells drawn

[P] - Correct shapes and proportions of the different tissue layers drawn

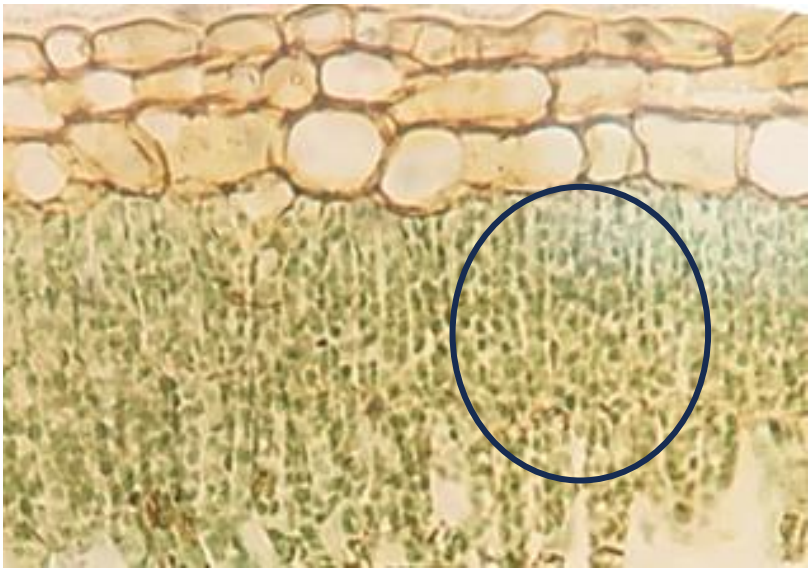
[D] - Accurate representation of at least 4 layers of tissue, inclusive of either vascular bundle and / or hairlike projection layer

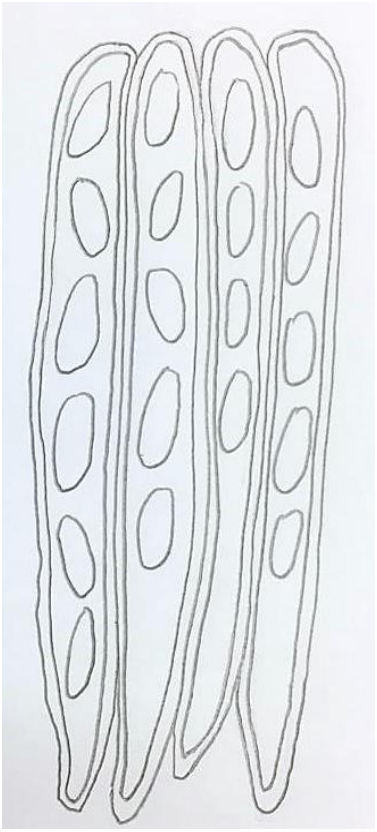
(ii) Observe the cells of the leaf on **S1** that are directly involved in photosynthesis.

Select **four** adjacent cells that are arranged in a line.

Each cell must touch at least one other cell.

Make a large drawing of this line of **four** cells.





[3]

Marks Allocation

[S1] - Size of drawing at least ¾ of space given; Lines continuous and smooth;

[D] - 4 adjacent cells with chloroplasts (adjacent cells must be touching one another, joined end to end

[P] - Cells of correct shape with appropriate proportion of cell wall thickness, wrt cell size; cell wall shown as 2 thin uniform double lines

(b) Fig. 3.2 is a photomicrograph of a stained transverse section through a leaf of a different type of plant.

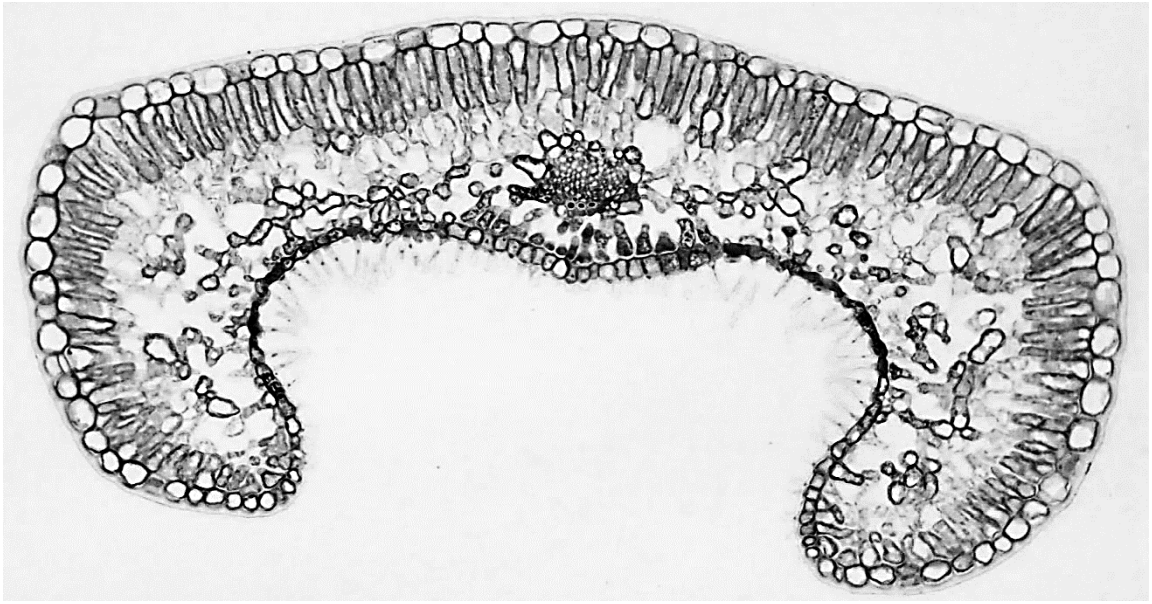


Fig. 3.2

The presence of a thick waxy cuticle in the leaf section in Fig. 3.2 supports the conclusion that the plant is adapted to live in a hot and arid environment.

Observe the leaf section in Fig. 3.2.

(i) Suggest and explain **one** other observable feature in the section in Fig. 3.2 which supports this conclusion.

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[1]

[Any 1]

1. **Hairlike structures** on the underside of the leaf + **trap water vapour** near leaf and hence reduce transpiration;
2. **Leaf is rolled up** + to **reduce exposure of stomata to the air** and hence evaporative water loss;

(ii) Plants with the same xerophytic features in their leaves seen in Fig. 3.2 are abundant in hot and arid environments.

Suggest how the abundance of these plants supports Darwin's theory of natural selection.

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[3]

1. ref. dry/arid climate/environment / lack of water, as the selection pressure;
2. Plants with leaves having (any of the above features in (a)) **being selected for, able to survive better till maturity to reproduce, and pass on alleles for these traits to their offspring;**
3. Leading to **increase in allele frequency** for the above trait in the gene pool/population (OWTTE);

(c) Fig. 3.3 is a photomicrograph of the same leaf section that is in Fig. 3.2.

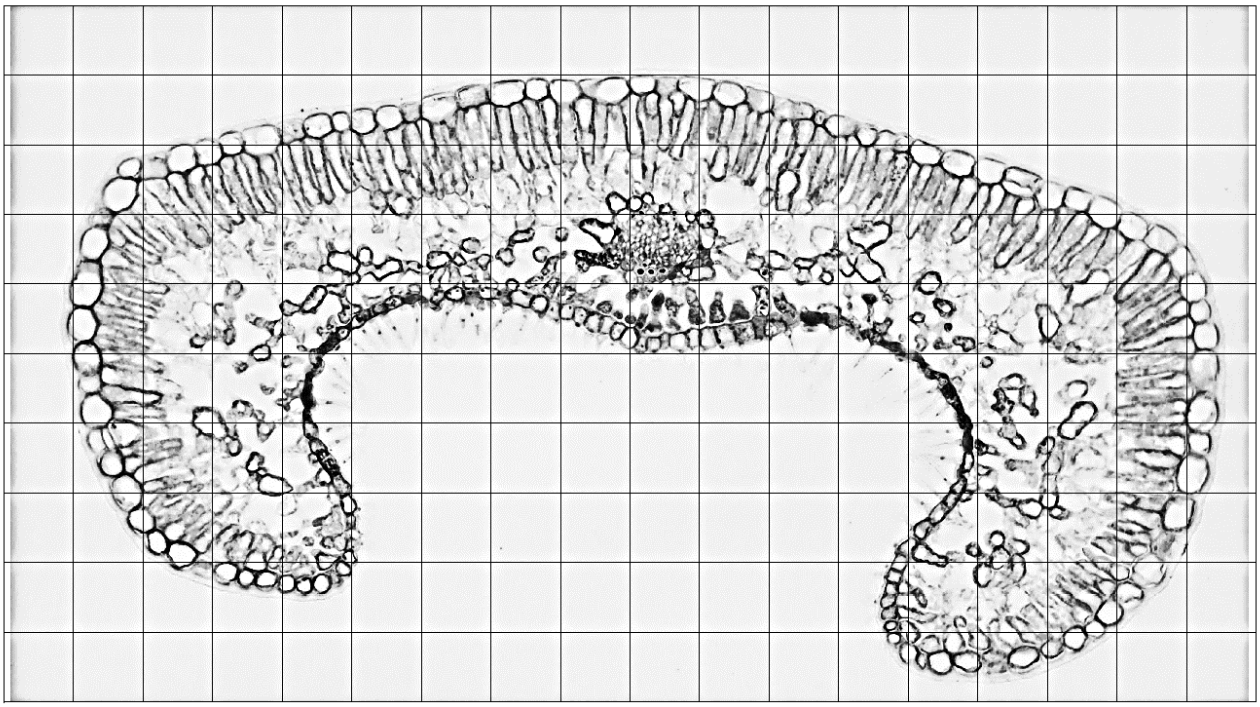

Fig. 3.3

You will need to use the grid to estimate the area of the palisade layer (layer of cells found below the upper epidermis of the leaf) **and** the total area of the leaf section in Fig. 3.3.

Each square of the grid is 1.0 cm^2.

In some squares the palisade layer or the leaf section does not fill the whole square.

(i) Describe the method you will use to decide which of these squares to include.

……

……

……

[1]

Count the number of squares with half or more than its area covered by the leaf region;

(ii) State the area of the palisade layer and the total area of the leaf section in Fig. 3.3.

area of palisade layer = 28 – 30 cm^2

total area of the leaf section = 77 - 78 cm^2

[2]

(iii) Suggest how you could modify the procedure you have used in **(c)(i)** to give a more accurate estimate of the area of the leaf.

……

……

……

[1]

1. Use a grid / graph paper with squares of area less than 1 cm^2;
2. Further divide out the grid into smaller squares, count using same method

[Total:15]