H2 ANDERSON SERANGOON JUNIOR COLLEGE
HIGHER 2

CANDIDATE

CLASS

CLA
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BIOLOGY 9744/04

Paper 4 Practical **28 August 2024 Wednesday**

Candidates answer on the Question Paper. **2 hours 30 minutes**
Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your name and class 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 an 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	55

This document consists of **21** printed pages and **3** blank pages.
Answer **all** questions.

1 Bromelain is a proteolytic enzyme, which catalyses the hydrolysis of proteins into amino acids. This enzyme can be found in fruits, such as pineapple.

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

You will investigate the effect of different concentrations of pineapple bromelain on gelatin breakdown.

You are required to:

- make five boiling tubes of gelatin gel
- obtain pineapple juice extract using the pineapple sample provided
- prepare a range of pineapple bromelain concentrations
- add each concentration of pineapple bromelain solution into a boiling tube of gelatin gel
- measure volume of liquified gelatin after 10 minutes of incubation.

You are provided with:

- 1 pineapple sample of a fixed mass, in a petri dish labelled **A**
- 100.0 cm^3 distilled water **W**, in a container labelled **W**
- 3.0 g gelatin powder, in a specimen tube labelled **G**.

Read steps **1- 6**.

Proceed as follows.

1 Using the container labelled **hot water**, collect approximately 100 cm^3 of hot water from where it is provided in the laboratory.

2 Add all of the gelatin powder in a specimen tube labelled **G** into a beaker labelled **X**. Pour 75.0 cm^3 of hot water directly into beaker **X**.

3 Use a glass rod to mix gently, so as to minimise air bubbles from forming. Stir for at least 1 minute to allow the powder to dissolve completely to form a gelatin mixture.

4 Take up 10.0 cm^3 of the gelatin mixture into a syringe.

5 Position the syringe so that the nozzle touches the side of one of the boiling tubes.

6 Gently push the plunger of the syringe so that the mixture runs slowly down the side of the boiling tube to the bottom.

7 Repeat step **6** for the remaining four boiling tubes.

8 To the beaker labelled **ice-bath**, add ice to approximately the 400 cm^3 mark. Immediately, place all five boiling tubes into the beaker of ice. Ensure all tubes are positioned upright and incubate in the ice bath for at least 10 minutes to allow setting of the gelatin.

During this incubation period, continue with step **10**.

(a) **(i)** Explain why it is necessary for the tubes to remain upright throughout the cooling period.

so that gelatin solution remains **evenly distributed** along the length of the tube/ **even surface** formed after gel is set → allows **surface area to volume ratio** to remain **constant**, since differences in surface area of gelatin can affect gelatin breakdown by bromelain enzyme. [1]

9 After 10 minutes, check the consistency of the gelatin by tilting the mixture slightly. If the gelatin does not move, the gelatin gel is set. Otherwise, continue to incubate in the ice- bath for another 5 minutes.

10 Transfer the pineapple sample in the petri dish labelled **A** onto a white tile. Using a kitchen knife, cut the pineapple sample into small pieces.

11 Using a plastic spoon, add the small pieces of pineapple into the mortar. Add 6.0 cm^3 of distilled water **W** and crush the pineapple pieces using the pestle for 1 minute to form a mixture.

12 Use a plastic spoon to transfer the first half of the pineapple mixture onto a sieve to extract the pineapple juice. Using the pestle, grind the pineapple mixture on the sieve for 1 minute. Collect the pineapple filtrate **F1** in the beaker labelled **B**.

13 Use a plastic spoon to transfer the pineapple mixture left on the sieve into the petri dish labelled **A**.

14 Repeat step **12** for the remaining half of pineapple mixture in the mortar.

Before proceeding to step **16**, you will need to obtain at least 10.0 cm^3 filtrate. If less than 10.0 cm^3 filtrate is obtained, proceed to step **15**.

15 Use a plastic spoon to transfer the pineapple mixture on the petri dish from step **13** and pineapple mixture left on the sieve from step **14** to a filter bag. Squeeze the filter bag using your fingers to collect the pineapple juice in the beaker labelled **B**

16 You will carry out a serial dilution of pineapple filtrate **F1** to reduce the concentration of pineapple bromelain solution by a factor of 2 between each of four successive dilutions to obtain **F2**, **F3**, **F4** and **F5**.

You are required to make a sufficient volume of each pineapple bromelain solution so that, once the serial dilution has been completed, there is a volume of at least 5.0 cm^3 for each concentration prepared.

Assume that the filtrate **F1** obtained is considered pure pineapple juice.

(ii) Complete Table 1.1 to show how you will make the concentrations of pineapple bromelain solutions **F2**, **F3**, **F4** and **F5**.

Table 1.1

	pineapple bromelain solution				
	F1	**F2**	**F3**	**F4**	**F5**
percentage concentration of pineapple bromelain solution	**100**	**50.0**	**25.0**	**12.5**	**6.25**
percentage concentration of pineapple bromelain solution to be diluted		**100**	**50.0**	**25.0**	**12.5**
volume of pineapple bromelain solution to be diluted/ cm^3		**5.0**	**5.0**	**5.0**	**5.0**
volume of distilled water **W** to make the dilution/ cm^3		**5.0**	**5.0**	**5.0**	**5.0**

[3]

1. Correct **percentage concentrations** of pineapple bromelain solution (**3 sf**)
2. Correct **percentage concentrations** of pineapple bromelain solution to be diluted (**3 sf**)
3. Correct **volumes** of pineapple **bromelain solution and W** (**1 dp**)

17 Prepare pineapple bromelain solutions **F2**, **F3**, **F4** and **F5** in the plastic containers provided, as shown in Table 1.1.

18 Once the gelatin is set, label the different boiling tubes as **F1**, **F2**, **F3**, **F4** and **F5**. Place the labelled boiling tubes of gelatin on the test-tubes rack.

19 Add in 4.0 cm^3 of pineapple bromelain solution to each of the five tubes, **F1**, **F2**, **F3**, **F4** and **F5**, respectively. Start timing immediately.

20 At the end of 10 minutes, pour the liquified gelatin with the pineapple bromelain solution into a measuring cylinder carefully and measure the volume collected.

21

(iii) Record your results in an appropriate table.

Percentage concentration of bromelain solution	Volume of liquified gelatin collected / cm^3
100	4.5
50.0	4.3
25.0	4.1
12.5	4.0
6.25	4.0

1. Results presented in a **proper table drawn with ruled lines**
2. Correct **headings with units**: **Percentage concentration of bromelain solution** and **Volume of liquified gelatin collected / cm^3**
3. Results for **all** percentage concentrations stated in (iii) recorded
4. Correct **precision of data** – volume to **1 dp** and percentage concentrations to **3 sf** [4]

Trend is not marked

(iv) One source of error in the method is the difficulty in maintaining the boiling tubes upright in the ice-bath during the cooling period.

State **two other** significant sources of error in this investigation **and** suggest how each of these errors can be improved.

1. State: **Temperature of set up** was **not controlled** throughout the experiment/ temperature of gel was low at the start of the reaction → enzyme inactivated → low rate of reaction
2. Suggestion: Use a **thermostatically-controlled** water bath.
3. When pouring the liquid gelatin into the measuring cylinder, **small pieces of gelatin may drop into the cylinder**, causing inaccurate volumes to be recorded.
4. Use a **sieve** during the transfer of liquid gelatin, so that only liquid gelatin is collected in the measuring cylinder *(reject filter paper which absorbs liquid)*
5. Unable to **standardise the duration** of experiment
6. Perform **each reaction individually**/ stagger the start of each reaction at a **fixed time interval** eg 1 minute [4]

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

1. Replace the bromelain solution with **equal volume** **(or stated volume of 4 cm^3)** of **boiled and cooled** bromelain solution and subject control setup to the same experimental conditions or variables

[1]

(b) Pineapples are sweet as they contain a large amount of reducing sugars such as glucose and fructose. A fruit seller claimed that a new variety of pineapple, MD, is the sweetest among other varieties such as Red Spanish.

You are provided with:

- pineapple juice from MD variety, in a container labelled **J1**
- pineapple juice from Red Spanish variety, in a container labelled **K**
- Benedict's solution, in a container labelled **Benedict's solution**.

You are required to:

- carry out reducing sugar test on pineapple juices **J1** and **K**
- use the results to determine which pineapple juice, **J1** or **K**, is sweeter.

Benedict's solution is harmful. Suitable eye protection should be worn. If Benedict's solution come into contact with yourskin, wash off immediately under cold water.

Use the beaker or container labelled **hot water** to collect approximately 400 cm^3 of hot water from where it is provided in the laboratory.

Read steps **1- 9.**

Proceed as follows.

1 Set up a water-bath using the hot water provided and the beaker labelled **water-bath.** Heat the water to boiling, ready for step **6**.

To test for reducing sugar:

2 Put 2.0 cm^3 of **J1** into an appropriately labelled test-tube.

3 Put 2.0 cm^3 of Benedict's solution into the same test-tube.

4 Shake gently to mix the contents.

5 Repeat steps **2 - 4** for **K**.

6 Place the two test-tubes in the boiling water-bath. Start timing immediately.

7 After 2 minutes, carefully remove the tubes from the boiling water-bath and place them in a test-tube rack.

8 Make sure that the Bunsen burner is switched off.

9 Record your observations of the contents of the test-tubes.

(i) Complete Table 1.2 by recording your results.

Table 1.2

pineapple juice	observations of contents of test-tubes
J1	Accept colour (red, brick red, orange-red) reject blue, blue-green, green, yellow
K	Accept colour (red, brick red, orange-red) reject blue, blue-green, green, yellow

[1]

(ii) Use your results to determine which sample of pineapple, **J1** or **K**, is sweeter.

pineapple juice - based on students' observations [1]

(c) Other than reducing sugars, pineapples also contain large quantities of ascorbic acid (vitamin C). Ascorbic acid is water-soluble and can pass through any selectively permeable membrane, such as a Visking tubing.

A student wanted to investigate the diffusion of ascorbic acid from pineapple extract **P** across a Visking tubing. Extract **P** was added into the Visking tubing. The ends of the Visking tubing were tied to form a bag, which was then placed in a boiling tube with distilled water.

To determine the concentration of ascorbic acid in a solution, the student used an indicator DCPIP. DCPIP reacts with ascorbic acid in a sample and becomes colourless, as shown in Fig.1.1. The higher the volume of DCPIP added to reach end-point, the higher the concentration of ascorbic acid in the solution.

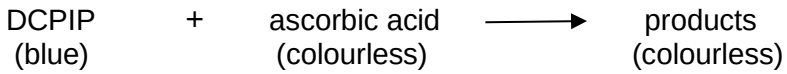


Fig. 1.1

The student performed some preliminary tests and found that DCPIP should be added one drop at a time using a syringe.

Design an experiment to investigate the rate (in cm^3 per min) at which ascorbic acid from pineapple extract **P** diffuses into the water surrounding a Visking tubing over a period of 10 minutes.

In your plan you must use:

- DCPIP indicator
- syringe
- stopwatch.

Assume you are provided with a Visking tubing containing 6.0 cm^3 of pineapple extract **P** in a beaker containing a fixed volume of distilled water. **You do not need to include details of how to set up the Visking tubing.**

You may select from the following apparatus and plan to use appropriate additional apparatus:

- normal laboratory glassware, e.g. test-tubes, beakers, glass rods, etc.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- be illustrated by relevant diagrams, if necessary
- identify variables you will need to control
- identify the colour of solution when the end-point is reached
- use the correct technical and scientific terms
- include layout of results tables and graphs with clear headings and labels.

You can consider all steps in the procedure to be low risk and there is therefore no need to include reference to any safety measures in your plan.

For students' reference, no marks awarded

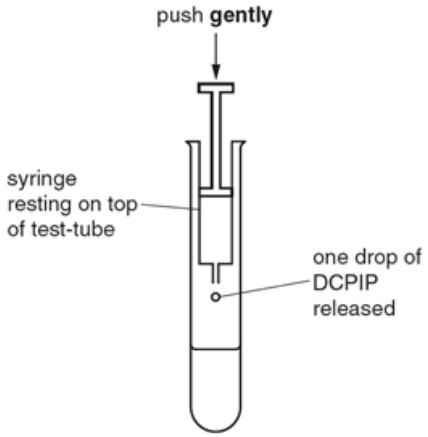
*[**V:** Variables with units stated];*
***Independent variable**: Time interval at which water sample is taken out from boiling tube /min (2, 4, 6, 8 10 / minutes)*
***Dependent variable**: Volume of DCPIP to reach end point /cm^3*

VC: Variables to be kept constant [1 mark each, max 2]:

1. Volume of water removed for testing -- Using a 5cm^3 syringe to remove a **fixed volume of water / sample (i.e. 3cm^3)** for testing;
2. Volume of DCPIP used i.e. , 5cm^3 of DCPIP added using a syringe, accept **any volume less than 5cm^3** since a 5cm^3 syringe is used, *AVP depends on the syringes students chose to use*;
3. **Incubation temperature of Visking tubing** in beaker of distilled water, maintained in **thermostatically controlled water bath**

[**Procedure 1:** Correct description of extracting water sample for testing – 1 mark each]
1. Immediately **start timing** using the **stopwatch** and **remove the first sample of water after 2 minutes**.
2. Using a **syringe**, transfer **3cm^3 of this water sample** *(accept any volume less than **5cm^3**)* into a test-tube.

[**Procedure 2**: Correct procedure + description of determination of endpoint – 1 mark each]
3. Fill a **syringe** with **5 cm^3 of DCPIP**. *(award only once), volume used should be the same as VC MP2*
4. Wipe off any drops of DCPIP from the outside of the syringe with a paper towel.
5. Add one drop of DCIPIP to the mixture in the test-tube. **Mix gently** and **continue adding drops to observe colour change**, one at a time/ accept idea of **doing titration**;
6. until the **blue colour remains/ (does not disappear)**, which is the **end-point**



[**Data:** Measurement and manipulation of results – 1 mark each]
7. Record the **volume of DCPIP/ calculating volume of DCPIP (subtracting final from initial volume,** recorded by reading off the measuring cylinder**)** needed to **reach the endpoint**. (reject number of drops of DCPIP)
8. Repeat steps 4 -9 using samples taken from the **water surrounding the Visking tubing** at the **4min, 6min, 8min and 10min intervals.**

   [Calculating the rate of diffusion – 1 mark]
9. Determine the **rate of diffusion** (in cm^3 per minute) by calculating the **gradient** of the graph with x axis (time interval) and y axis (volume of DCPIP)

10. Volume of DCPIP is **proportional** to concentration of ascorbic acid in sample of water taken out.

[**R & R:** Repeats and replicates – 1 mark each]

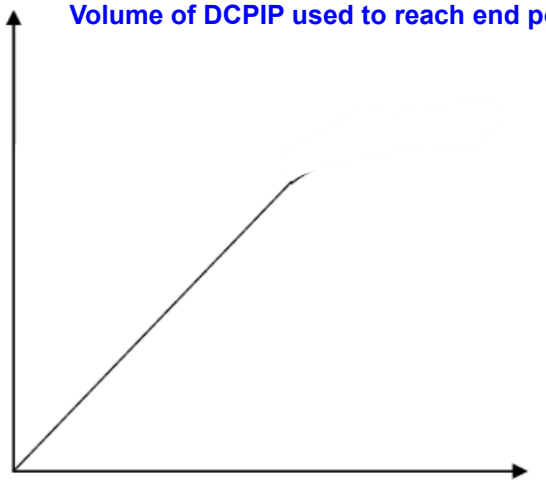
11. Perform **at least 2 more replicates** for each time interval and calculate the **average volume of DCPIP**, to ensure **reliability and reduce errors.**
12. **Repeat** the whole experiment **two more times** using **freshly prepared reagents and apparatus**, to ensure **reproducibility.**

[Table of results – 1 mark]

Time interval at which water sample is taken out from boiling tube /min	**Volume of DCPIP used to reach end point /cm^3**
2	
4	
6	
8	
10	

[8]

[Graph – 1 mark] Accept graph plateaus off



VC – 2 marks max
P1 + P2 – 3 marks max
Data – 3 marks max
R & R – 1 mark max
Results – 1 mark
Graph – 1 mark
Diagram – accept only when well-labelled – 1 mark

(d) Ascorbic acid is known to have antimicrobial properties. A scientist carried out an investigation to determine the effect of ascorbic acid on the growth of a species of Bacterium, *Bacillus subtilis*.

The growth of bacteria was investigated by measuring the mass of the bacteria when grown on agar containing different concentrations of ascorbic acid.

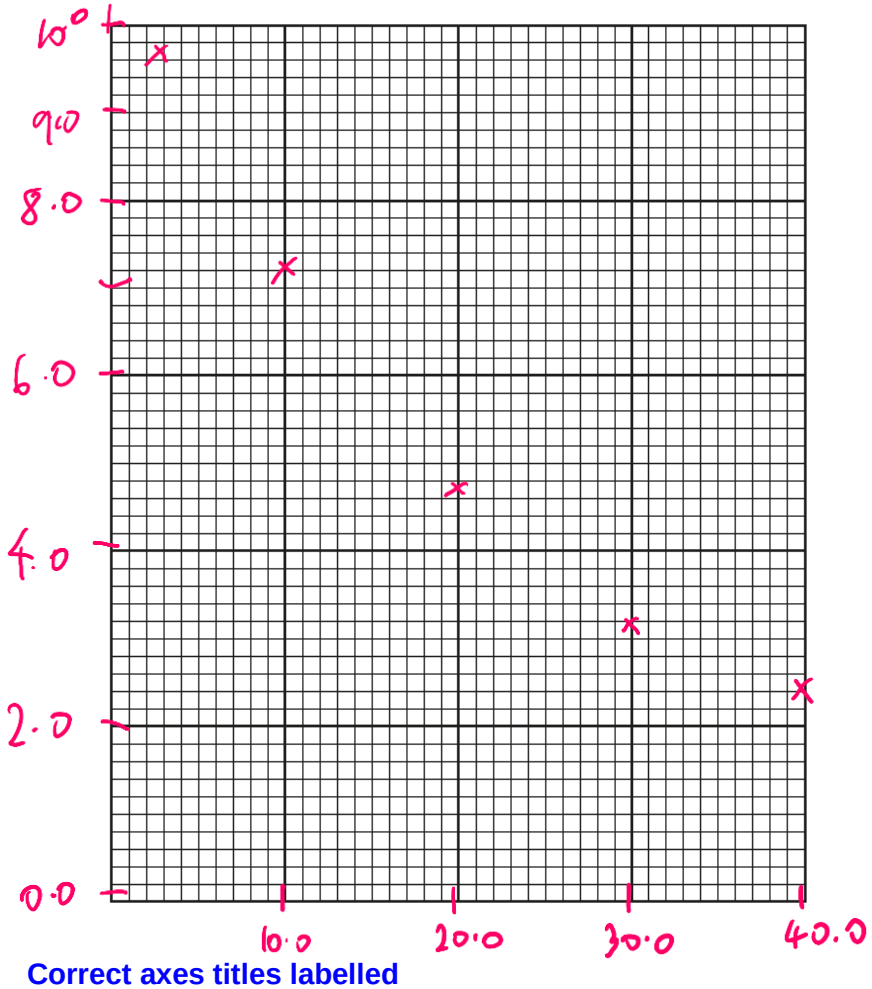
All other variables were kept constant.

The results are shown in Table 1.3.

Table 1.3

ascorbic acid concentration / mM	mass of *Bacillus subtilis* / mg
2.5	9.7
10.0	7.2
20.0	4.7
30.0	3.1
40.0	2.4

(i) Plot a graph of the data shown in Table 1.3 on the grid provided.



1. Correct axes titles labelled

- x-axis – ascorbic acid concentration / mM and
- y axis – mass of *B. subtilis* / mg

2. Correct **plotting of all five points** using small crosses or dots in circles;

[4]

3. **Appropriate scale and axes intervals labelled**
   - Appropriate scale that covers at least ½ of the grid;
   - **Axes intervals labelled at least every 10 squares**. **Correct d.p** following values in table
     - x axis – 10 mM every 10 squares
     - y axis – 2.0 mg for every 10 squares

4. **Best fit graph**
   Clean, thin line drawn from **point-to-point, must pass through all 5 plotted points using RULER,** or **best-fit curve**; **no extrapolation beyond plotted points**

(ii) Suggest the effect of ascorbic acid on bacteria cells.

1. Describe and quote data;
   Any 1 below
2. Acidic pH **denature enzymes**/proteins involved in **bacteria cell division/binary fission/growth**;
3. Extreme pH changes can affect the proton gradient across the bacterial cell membrane, disrupting ATP synthesis;
4. inhibit enzymes involved in bacterial metabolism/any named cellular processes/ eg DNA replication, protein synthesis

[2]

[Total: 29]

2 (a) Fig. 2.1 is a photomicrograph of a stained transverse section through a leaf of a pineapple plant (*Ananas comosus*).

You are not expected to be familiar with this specimen.

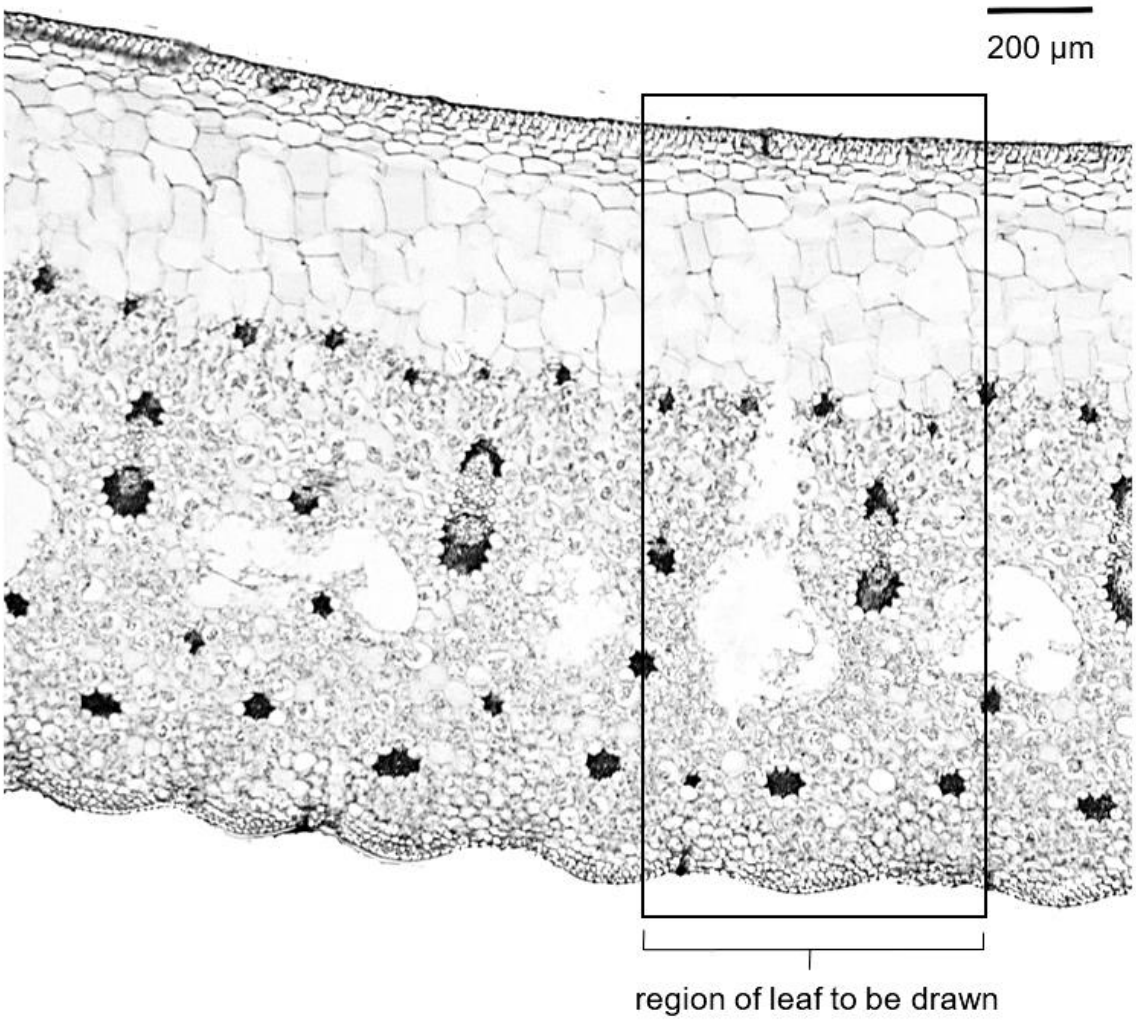


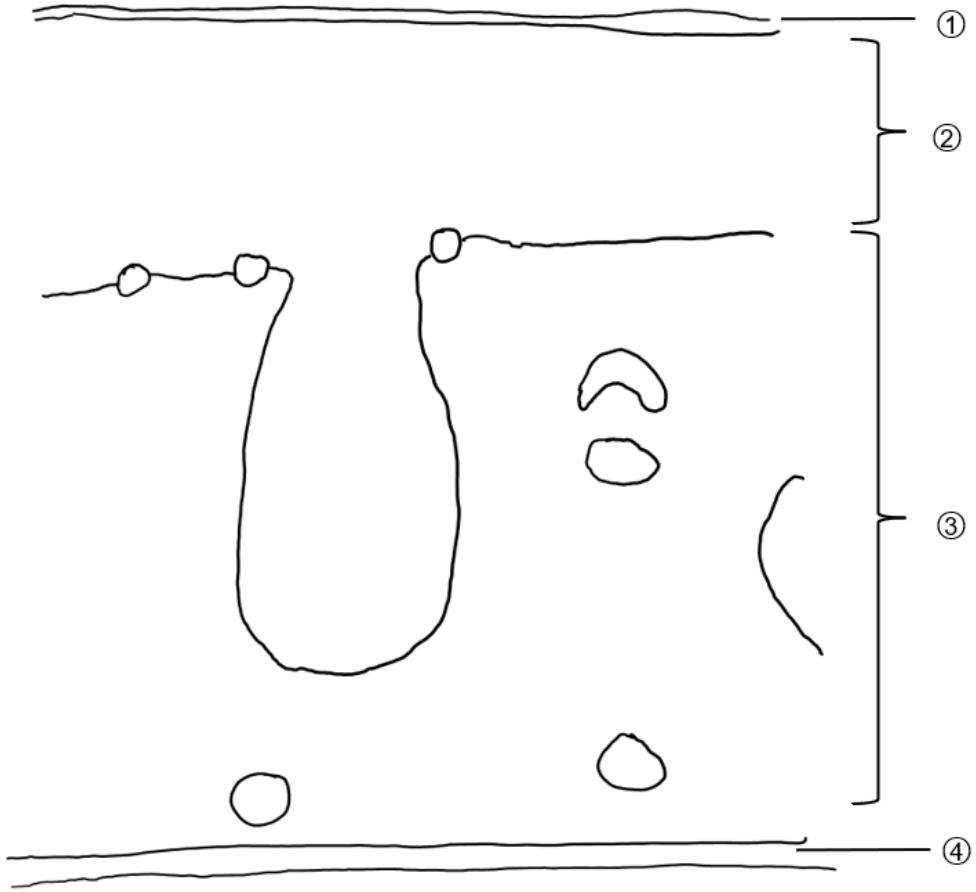
Fig. 2.1

(i) Draw a plan diagram of the region of the leaf indicated by the box on Fig. 2.1.

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

No cells should be drawn.

Labels are **not** required.



PDO

- No cells + clear continuous lines + no side lines;
- Correct size of plan drawing + no labels;
  Width more than 7 can
  Length more than 6 cm

MMO

- Correct arrangement of tissues + position of air space (ignore shape);
- Correct shape of large air space and vascular bundles (accept circles or spiky shape);

[4]

(ii) You can assume that the actual length of the bar in Fig. 2.1 is 200 μm.

Use this information to calculate the magnification of your drawing in **(a) (i)**.

Show all the steps in your calculation.

- Determine actual length of sample + indication on Fig. 2.1 (can use either length or width)
- Correct conversion to same unit as length of scale bar (μm) + calculation of magnification in whole number;

magnification = x…… [2]

(b) Pineapple plant (*Ananas comosus*) grows under strong sunlight and has one consistent type of leaves. However, a student noticed that the leaves on a different plant species growing close to a wall had two types of leaves.

The leaves next to the wall were in the shade while the leaves on the side away from the wall were exposed to the sun. The shape of the two types of leaves and the length of the internodes on the stem also looked different and are shown in Fig. 2.2 and Fig. 2.3 respectively.

The student decided to investigate these differences by measuring some features of 30 leaves and internodes from each side of the plant.

Fig. 2.2 shows the leaf shape

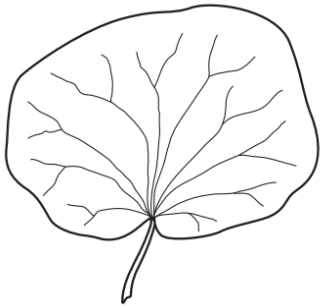


Fig. 2.2

Fig. 2.3 shows an internode

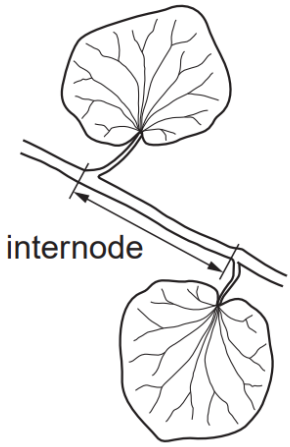


Fig 2.3

Table 2.1 shows the student's results, including the standard deviation.

Table 2.1

	shaded leaves	**exposed leaves**
mean internode length / mm	23 ± 4	15 ± 3
mean surface area of leaves / mm^2	2750 ± 12	1800 ± 15
mean dry mass of leaves / mg	50 ± 8	60 ± 10
mean leaf surface area : leaf mass ratio	55 ± 9	30 ± 6

(i) Describe how the student obtained the independent variable being investigated.

- Differences in light exposure of the two types of leaves (in terms of intensity or duration)/ leaves under different light intensity or exposure *(do not allow light unqualified or position in shade / sun)*
- Systematic way of obtaining the leaves e.g. 3rd leaf from the apex / different heights / all from the same height / equal light exposure for equal duration
- 30 leaves for each light intensity and 30/15 internodes (1 internode between 2 leaves)

[2]

(ii) Complete Table 2.2 to describe how the student could measure the following variables in the investigation.

Table 2.2

variable	description of how the variable could be measured
surface area of leaf	method of measuring surface area; • e.g. draw round each leaf on grid or use transparent grid over leaf / measure diameter(s) of leaf using ruler • count squares / use formula πr^2 (because the leaves is circular in shape) • both sides needed to get total surface area;
dry mass of leaves	• e.g. digital balance / weighing scales to weigh • e.g. sample leaves dried in oven/ dehydrator until mass constant (accept under the sun)

[4]

(iii) Explain why the mean surface area of the shaded leaves is larger than that of the exposed leaves.

- (mean surface area of the shaded leaves is larger) because more surface area to pack for mesophyll cells, thus more chloroplasts
- The larger surface area helps shaded leaves maximize light absorption / capture more light in low-light conditions.
- (In contrast, leaves exposed to direct sunlight are often smaller, smaller surface area) to prevent water loss and reduces the risk of damage from excessive light.

[2]

(c) The student carried out *t*-tests for leaf surface area: leaf mass ratio and for internode length.

The formula for *t*-test is

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

key to symbols

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

(i) Complete the calculation to find the value of *t* for the internode length.

Show your working.

$t = \frac{-}{\sqrt{\frac{4^2}{30} + -}}$ $t = \frac{23 - 15}{\sqrt{\frac{4^2}{30}} + \frac{3^2}{30}}$;

$= \frac{}{0.9}$ $\frac{(8)}{(0.9)} = 8.9$;

ignore any working in the answer
allow 9 / 8.89 and 8.88

t = ... [3]

Table 2.3 shows the critical values at p = 0.05 for the *t*-test.

Table 2.3

degrees of freedom	18	20	21	22	23	24	25	26	27	28	29	30	40	60	∞
Critical value	2.10	2.09	2.08	2.07	2.06	2.06	2.06	2.06	2.05	2.05	2.04	2.04	2.02	2.00	1.96

(ii) The number of degrees of freedom is 58.

State how the number of degrees of freedom was calculated.
total number of measurements –1 for each set of measurement /
(30 – 1) + (30 – 1) = 58

2n – 2 / (n – 1) + (n – 1)
60 – 2 = 58 [1]

(iii) The leaf surface area: leaf mass ratio gave the value of ***t* = 12.6**.

Use Table 2.3 and your answer in **(c) (i)** and **(ii)** to decide whether the null hypothesis of no differences between the two types of leaves, should be accepted or not.

Explain your answer.

- both calculated t values (The leaf surface area: leaf mass ratio ***t-value* = 12.6** and the internode length ***t-value* = 8.9**) are greater than the critical value
- critical value is between 2 and 2.02/ 2 < t< 2.02 *(reject 2.02 < t < 2.00)*
- both results are significant / not due to chance / caused by another factor / light exposure; [2]

[Total: 19]

3 During this question you will require access to a microscope and slide **M1**.

Slide **M1** is a stained transverse section of a leaf from a different type of plant.

You are not expected to be familiar with this specimen.

(a) (i)

- Observe the slide with a microscope and select the area that shows a small vascular bundle and a large vascular bundle next to each other, as shown in Fig. 3.1.

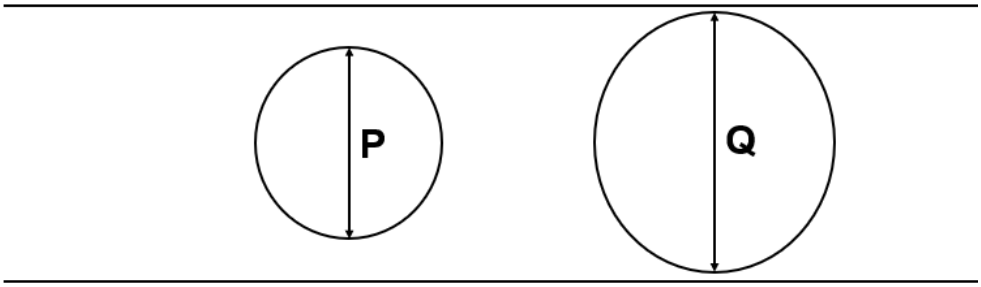


Fig. 3.1

- Measure **and** record the length of the two vascular bundles in eyepiece graticule units.

length of small vascular bundle (**P**) …… eyepiece graticule units
x10 range (5-15epg)
x40 range (20- 45epg)
1m

length of large vascular bundle (**Q**) …….…… eyepiece graticule units
x10 range(10-25epg)
x40 range(60-75epg)
1m

- Record the magnification of the objective lens you used when measuring the length of the vascular bundles with the eyepiece graticule.

magnification = x …….. [2]
Accept x10/ x40 (no marks for this segment

(ii) Calculate the actual length of the two vascular bundles.

Show **all** the steps in your calculation, including the appropriate units.

- 1m for eyepiece calibration
- 1m to multiply by the calibration factor
  (X 10) 1 eyepiece is 10 µm/ (X 40) 1 eyepiece is 2.5 µm
- 1m for correct answer for both P and Q

actual length of small vascular bundle (**P**) …… µm

actual length of large vascular bundle (**Q**) …… µm [3]

(iii) Calculate the percentage difference in length between small vascular bundle and large vascular bundle.

Show your working and give your answer to **two** significant figures.

- shows the length of Q minus the length of P divided by the length of Q multiplied by 100/ accept divide by P also;
- records answer to **two** significant figures ;

percentage difference in length = ……..
[2]

[Total: 7]