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BIOLOGY
Paper 5
(YEARLY)

About A LEVEL

About **Learning Corner**

This section reveals the extra/relevant information that are not required as part of the model answers but to expand the student's knowledge in the respective fields. At the same time, it induces eagerness and interest into their learning process.

period	2010 to 2025
contents	June & November, P5 Worked Solutions
form	Year By Year
compiled for	A Levels
special features	Learning Corner

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CONTENTS

Question 1

Polyphenol oxidase is an enzyme involved in the browning of fruit.

Fig. 1.1 shows browning of the skin of bananas.

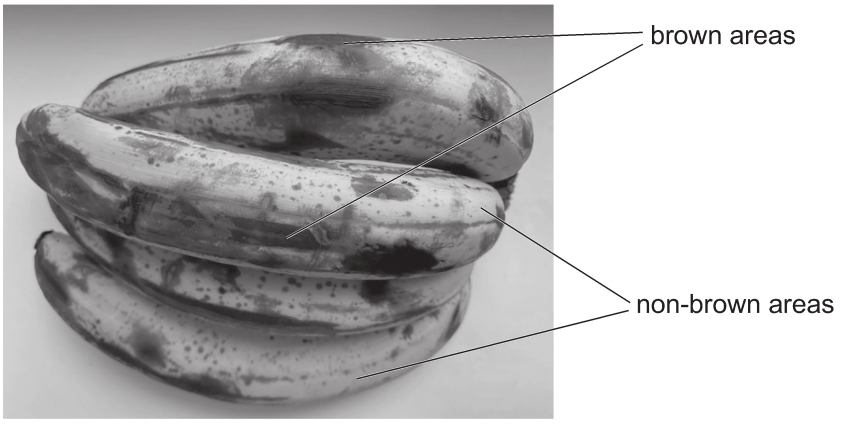

Fig. 1.1

A student extracted polyphenol oxidase enzyme from ripened bananas. The student used this enzyme extract to investigate the factors affecting the activity of the enzyme.

The student initially found that the enzyme extract was too concentrated, so the student made a 1:10 dilution of the enzyme extract.

(a) Describe how the student made a 1:10 dilution of the enzyme extract.

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..... [1]

(b) The student studied the effect of pH on the activity of polyphenol oxidase enzyme.

Dopamine hydrochloride solution was used as a substrate for the enzyme. The product of this reaction is red in colour.

A colorimeter can be used to follow the progress of this enzyme-catalysed reaction.

The student set up:

- 7 colorimeter tubes containing a solution of the substrate, dopamine hydrochloride
- 6 colorimeter tubes containing diluted polyphenol oxidase extract, each in a different buffered solution: pH 3.0, pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0
- 1 colorimeter tube containing water in pH 7.0 buffer.

The colorimeter tubes were equilibrated at a set temperature.

A colorimeter was set up with a filter transmitting light at 470 nm.

At time 0 seconds, the substrate solution and the polyphenol oxidase solution at pH 3.0 were mixed in one colorimeter tube. The colorimeter tube was then immediately put into the colorimeter.

The absorbance was measured at 15 second intervals over the first three minutes of the reaction. The procedure was repeated for the remaining buffered solutions and the water at pH 7.0.

(i) Identify the **independent** variable in this experiment.

..... [1]

(ii) Identify **one** variable that the student has standardised.

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..... [1]

(c) The student then plotted a graph of the results, showing the increase in absorbance. Absorbance is measured in absorbance units (au).

Fig. 1.2 shows the results for the increase in absorbance at **pH 5.0**.

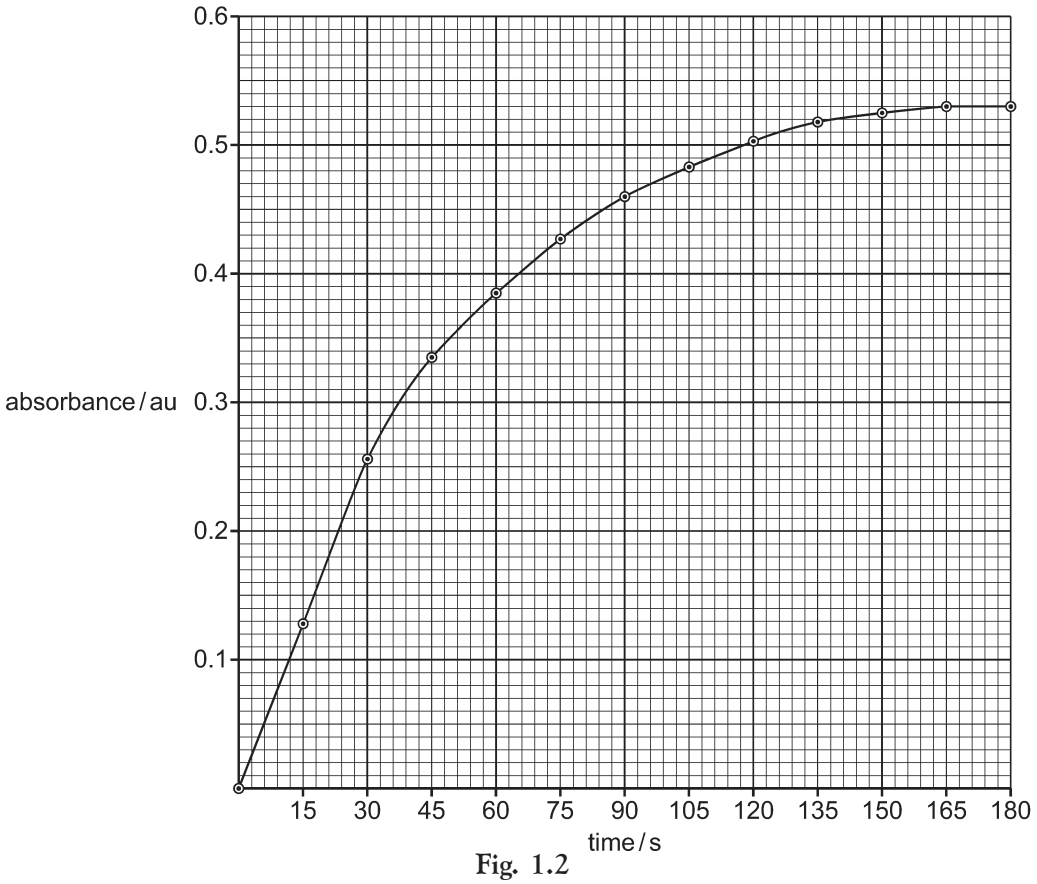

Fig. 1.2

(i) Use Fig. 1.2 to calculate the initial rate of this reaction in absorbance units per second (au s^{-1}). Show your working.

initial rate of reaction = ... au s^{-1} [2]

(ii) Using the internet, the student found that pH 7.0 is the optimum pH for polyphenol oxidase. On Fig. 1.2, sketch the line you would expect to get for the rate of reaction at **pH 3.0**. [1]

(d) The student wanted to determine the effect of the concentration of the substrate, dopamine hydrochloride solution, on the rate of the enzyme-catalysed reaction.

The student was provided with a stock solution of 0.2% dopamine hydrochloride solution and the diluted enzyme extract prepared in **(a)**.

Describe a method the student could use to collect the data that is needed to determine the effect of the concentration of dopamine hydrochloride solution on the rate of the enzyme-catalysed reaction.

Your method should be set out in a logical order and be detailed enough to let another person follow it.

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(e) The browning of fruit such as bananas can make them more difficult to sell. One way to prevent browning of bananas is to use an anti-browning agent.

The student searched the internet and found data from different scientific papers on the inhibitory effect of various anti-browning agents on polyphenol oxidase from different plants.

The student processed and ranked these data, as shown inTable 1.1.

Table 1.1

anti-browning agent	**percentage of maximum activity of polyphenol oxidase**	**plant source of polyphenol oxidase**
heated onion extract	17.0	potato
fresh onion extract	44.4	potato
ascorbic acid	60.0	sorrel
L-cysteine	70.0	sorrel
potassium sorbate	76.8	potato
citric acid	80.4	potato
EDTA	88.0	red poppy
no anti-browning agent (control)	100.0	potato

The student concluded that heated onion extract was the most effective anti-browning agent and that EDTA was the least effective.

Use the information to suggest why this conclusion may **not** be supported.

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..... [2]

(f) The student found that a sulfur-containing molecule in the heated onion extract acted as a non-competitive inhibitor to the enzyme polyphenol oxidase.

Fig. 1.3 is a graph showing how the substrate concentration affects the rate of polyphenol oxidase activity.

On Fig. 1.3 sketch the curve that shows the rate of activity for polyphenol oxidase treated with the heated onion extract.

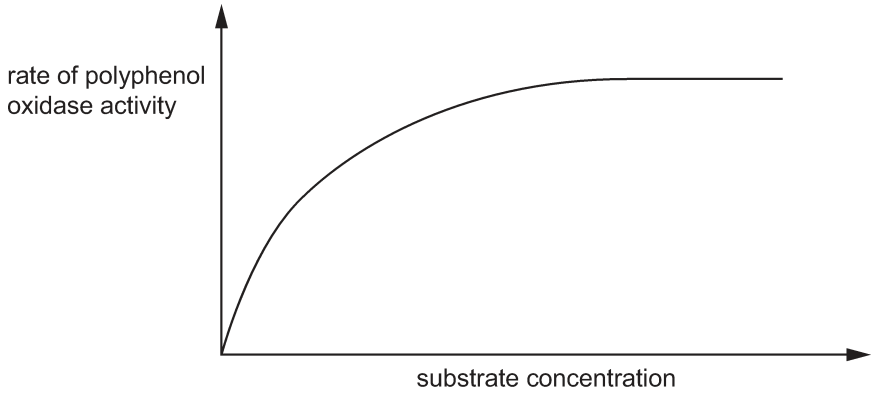

Fig. 1.3

[1]

[Total: 16]

Question 2

Air pollution due to motor vehicles is an environmental issue that needs constant monitoring.

Lichens consist of protoctists and fungi living in close association. The protoctists and fungi each benefit from this association. Lichens are found in a large range of habitats, including on the surface of tree trunks (tree bark), as shown in Fig. 2.1.

Fig. 2.1

Lichens are sensitive to air pollution. The abundance of lichens decreases with increasing levels of air pollution and so lichens can be used to monitor levels of air pollution.

Some scientists investigated the relationship between distance from a road and levels of air pollution. The scientists investigated this relationship by measuring the abundance of lichens on the tree trunks in a woodland beside a busy road.

The scientists used the grid shown in Fig. 2.2 to determine the abundance of lichens on the tree trunks.

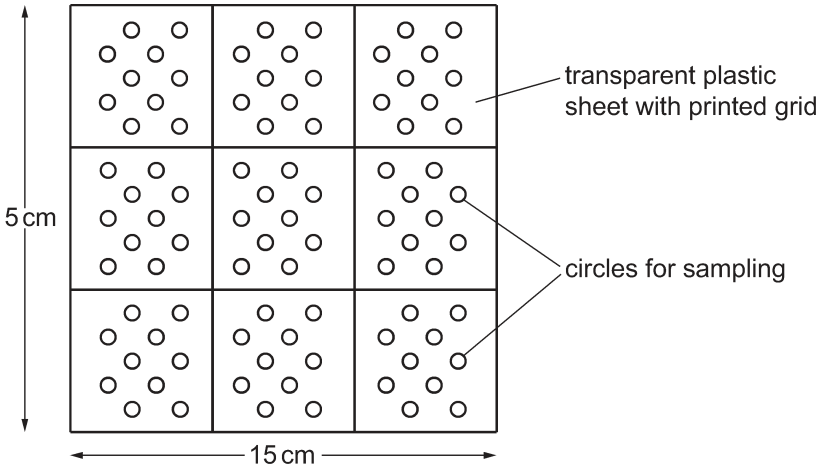

Fig. 2.2

For each tree sampled, the scientists:

- placed the grid against the tree trunk
- counted the number of circles that contained lichen
- used the number of circles that contained lichen to calculate the percentage cover of lichen on the tree
- measured the distance from the tree to the road.

(a) (i) State **two** variables that the scientists should standardise in this investigation.

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..... [2]

(ii) State **one** risk **and** the safety precaution that the scientists should take when measuring the abundance of the lichens on the tree trunks.

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..... [2]

(b) The scientists recorded the presence of lichens by shading in the circles on the grid where lichens were observed.

The results are shown in Fig. 2.3.

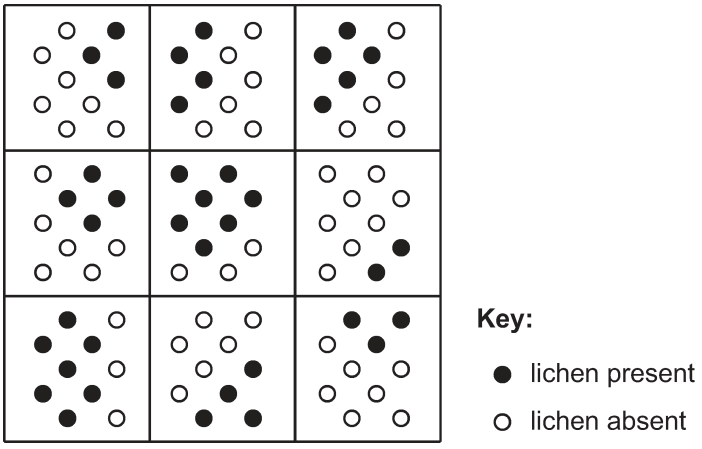

Fig. 2.3

Use the grid in Fig. 2.3 to calculate the percentage cover of the lichen on the tree trunk. Show your working.

percentage cover of lichen = ... % [2]

(c) The scientists measured the percentage cover of lichens on 10 trees, each growing at a different distance from the road.

The scientists carried out a Spearman's rank correlation analysis to find out if there was a correlation between the distance from the road and the percentage cover of lichens on the tree trunks.

Table 2.1 shows part of the table used to determine the data needed for the Spearman's rank correlation equation.

(i) Complete Table 2.1 for tree number 10. [1]

Table 2.1

tree number	**distance from road / m**	**rank for distance from road**	**percentage cover of lichen**	**rank for percentage cover of lichen**	**difference in rank (*D*)**	D^2
10	5.6	7	74	5		

The formula for calculating Spearman's rank correlation coefficient (r_s) is:

$$r_s = 1 - \left(\frac{6 \times \sum D^2}{n^3 - n} \right)$$

Key to symbols:

D = difference in rank between each pair of measurements

n = number of pairs of items in the sample

(ii) The scientists calculated the value of $\sum D^2$ as **39.75**.

Calculate the Spearman's rank correlation coefficient (r_s) value to **three** significant figures.

Use this space for working.

Spearman's rank correlation coefficient (r_s) = ... [2]

(iii) State the conclusions that can be made from the calculated value of Spearman's rank correlation coefficient (r_s).

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(iv) State how the scientists could use the calculated value of r_s to determine whether or not the correlation is due to chance.

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..... [2]

[Total: 14]

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Q1-Solution

(a) He added one part of polyphenol oxidase enzyme extract to nine parts of water or with buffer solution.

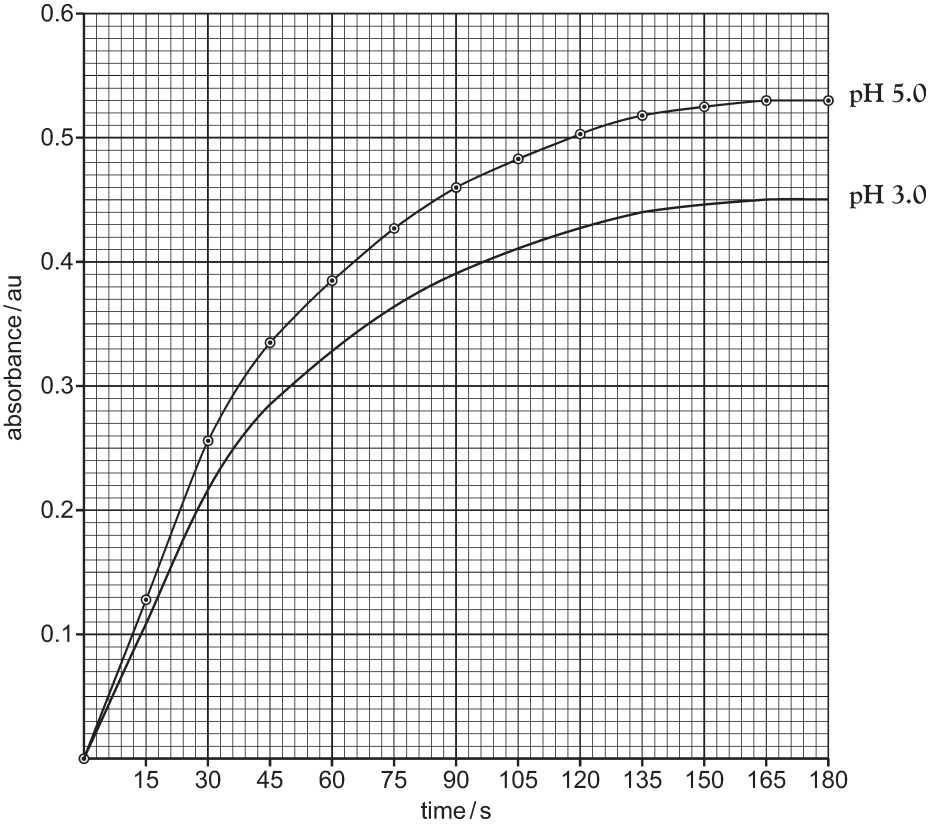
(b) (i) pH

(ii) Temperature of solutions in tubes to be kept same or constant. Optimum temperature set in water bath to be used.

(c) (i) 0.26 au and 30 s

$$\text{Initial rate of reaction} = \frac{0.26}{30} = 0.0087 \text{ au s}^{-1}$$

(ii)



(d) At least five dilutions, i.e., of 0.2% dopamine hydrochloride solution are made, which range from 0.0 to 0.2%, e.g., 0.0, 0.04, 0.08, 0.12, 0.16, 0.20. For making 0.04% dilution, 2 cm^3 of 0.2% solution is taken and 8 cm^3 of water is added. Similarly for 0.12% dilution, 6 cm^3 of 0.2% solution is taken and 4 cm^3 of water is added. Other dilutions are made according to proportionate dilution method in the same way. Tubes are

Answer Tips

(a) For example one cm^3 of enzyme extract added to 9 cm^3 of water.

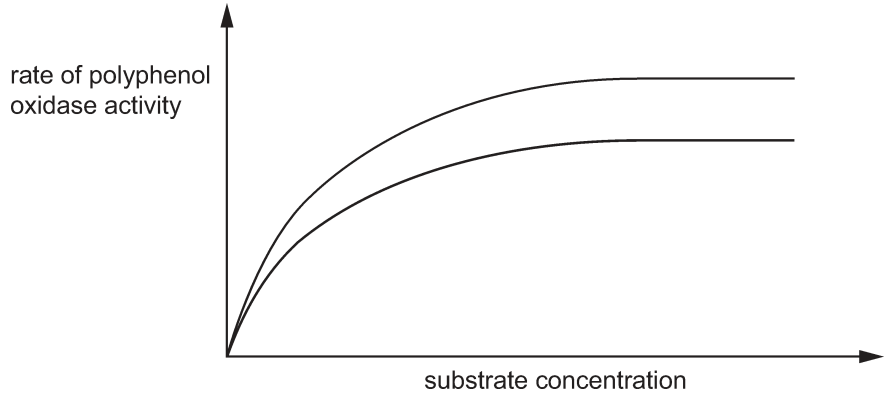
(b) (ii) Other variables are: wavelength (λ) of light filter of 470 nm, concentration of enzyme and total length of time or time intervals for taking reading.

(c) (i) Answer ranges between 0.0083 to 0.0089 or 8.3×10^{-3} to 8.9×10^{-3}.

kept in water bath set at 30 °C or incubator at this constant temperature. Enzyme and substrate solutions are separately kept at constant temperature for 5 minutes to equilibrate. Volume of substrate and enzyme is kept same for reaction. pH is also kept same. Colorimeter is set at zero and calibrated at the start and between each reading. Enzyme and substrate are mixed in different tubes and immediately kept in colorimeter. Immediately start timing. Measure absorbance at set time intervals for set time or record time at which red colour first appears. Three replicates or two repeats are done for same concentration and mean is calculated. Rate of reaction is then worked out by drawing a graph. Substrate is toxic, so don't drink or pour down the sink and wear gloves. Enzyme may be irritant causing allergy so use mask, gloves and goggles.

(e) Enzyme is used from different plant sources and not from bananas. Sources of information may be outdated or not reliable. No information is provided about standardised variables like temperature, pH, time intervals, volumes, concentrations etc. No statistical test or analysis is done.

(f)



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Answer Tips

(d) Light filter or wavelength of light may be kept constant as other variables. Colorimeter is equilibrated as blank or adding water to colorimeter tube. Dopamine hydrochloride is irritant and causes allergy. Buffer solution may also be irritant or causing allergy. As a control, water or boiled enzyme can be used.

(e) No information on what substrate is used or whether tested on bananas or other fruits. Type of inhibitor is not known (whether competitive or non-competitive). Other anti-browning agents may be more or less effective. Anti-browning agents should be tested on all sources of enzyme as they may act differently on other sources of enzyme.

Q2-Solution

(a) (i) Species or type of tree and its type of bark selected should be same. Grid should be kept at fixed height above the ground on tree trunk.

(ii) Tree or plant leaves or pollen from tree may cause scratches, allergy and irritation due to some toxins. One should wear glasses and gloves. Also some suitable medicine can be used and should not ingest the part of plant.

(b) Percentage cover of lichen $= \frac{39}{90} \times 100$

$= 43.3\%$

(c) (i)

tree number	distance from road / m	rank for distance from road	percentage cover of lichen	rank for percentage cover of lichen	difference in rank (*D*)	D^2
10	5.6	7	74	5	2	4

(ii) Spearman's rank correlation coefficient $(r_s) = 1 - \left(\frac{6 \times 39.75}{(10)^3 - 10} \right)$

$= 1 - \left(\frac{238.5}{990} \right)$

$= 1 - 0.241 = 0.759$

(iii) As $r_s = 0.760$, so it shows a strong correlation and positive correlation between both variables. Hence, it is showing that by increasing the distance from road also increases the percentage cover or abundance of lichens.

(iv) Calculated value of r_s is compared with critical values of r_s at $p = 0.05$ or less. If calculated r_s is greater than critical r_s value, then correlation is not due to chance.

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Answer Tips

(a) (i) Age or size of tree should be same. Grid to be placed horizontally.

(ii) Animals like insects or animal wastes may be found which are dangerous and can cause allergy or insects may bite, so work in groups and some specialist or expert should be accompanied. Fungi or lichens may be toxic or poisonous and one may inhale fungal spores which then cause infection. Hence, wear a mask. Nearby traffic on busy road may cause air pollution, so take precautions to wear mask and goggles.

(c) (iv) If calculated value of r_s is lesser than the critical r_s value, then correlation is due to chance.

Question 1

Tree plantations are areas where trees are planted for a particular purpose. Some tree plantations increase the supply of wood for construction and fuel. In many plantations, fast-growing, alien tree species are planted.

Undergrowth is found in and around plantations, increasing plant biodiversity. Undergrowth is mainly made up of indigenous (native) small plants and shrubs.

Fig. 1.1 shows part of a plantation of eucalyptus trees.

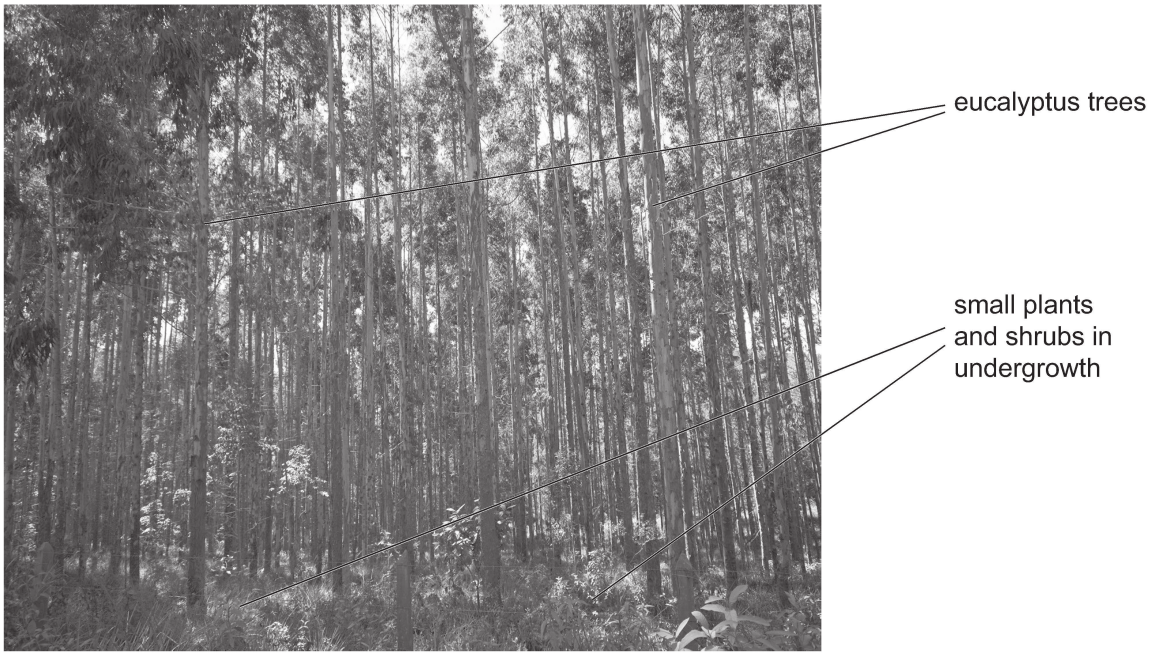

Fig. 1.1

Bangladesh has many tree plantations as part of a national tree-planting programme.

Two fast-growing, alien tree species planted in Bangladesh are acacia, *Acacia auriculiformis*, and eucalyptus, *Eucalyptus camaldulensis*.

Acacia and eucalyptus are considered to be invasive species as they outcompete indigenous tree species such as sal tree, *Shorea robusta*, and mango, *Mangifera indica*.

Scientists carried out an investigation into the biodiversity of plant species in the undergrowth in plantations of alien trees compared with the undergrowth in plantations of indigenous trees.

The hypothesis that the scientists tested was stated as:

The undergrowth in plantations of alien tree species will have a lower biodiversity of plant species than the undergrowth in plantations of indigenous tree species.

(a) (i) Identify the independent variable in this investigation.

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..... [1]

For the investigation, plantations were selected within the same region of Bangladesh with the same environmental conditions.

- Three plantations of each tree species were selected: acacia, eucalyptus, sal tree and mango.
- A plot measuring 36 m by 36 m was studied within each plantation.
- For each plot, belt transects were used to collect the data needed to determine Simpson's index of diversity (*D*).

For each type of plantation, Simpson's index of diversity (*D*) was calculated for the plant species in the undergrowth.

Each plot was studied in April, July and November.

(ii) Identify **two** variables the scientists standardised in this investigation.

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..... [2]

(b) The scientists used belt transects to investigate the undergrowth of each plot.

Describe how you could use belt transects to collect the data needed to determine Simpson's index of diversity (*D*) of the undergrowth in each of the plots.

Your method should be set out in a logical order and be detailed enough to let another person follow it.

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Table 1.1 shows the Simpson's index of diversity (*D*) values the scientists obtained for the different plantations during April, July and November.

Table 1.1

month	**Simpson's index of diversity (*D*) for undergrowth plant species in plantation**			
	acacia	**eucalyptus**	**sal tree**	**mango**
April	0.90	0.87	0.94	0.90
July	0.86	0.86	0.94	0.86
November	0.87	0.83	0.94	0.88
mean	0.88	0.85	0.94	0.88
±*s*	±0.02	±0.02	±0.00	±0.02

s = standard deviation

(c) State **and** explain whether the data in Table 1.1 support the hypothesis:

The undergrowth in plantations of alien tree species will have a lower biodiversity of plant species than the undergrowth in plantations of indigenous tree species.

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..... [1]

In China, a different group of scientists investigated whether the roots of invasive plant species produce chemicals that have an effect on the growth of other plants.

The scientists prepared an extract of the roots of staghorn sumac, *Rhus typhina*. Staghorn sumac is an invasive plant species.

The scientists prepared a stock solution of the root extract with a concentration of 10 mg cm^{-3}. The scientists then used the stock solution to prepare solutions with different concentrations of root extract.

(d) (i) Describe how the scientists could use the stock solution to prepare **50 cm^3** of solutions of the root extract with concentrations of 2.5 mg cm^{-3} and 7.5 mg cm^{-3}.

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..... [2]

In some regions of China, marigold plants, *Tagetes erecta*, are grown commercially.

In the investigation into the effect of staghorn sumac root extract on the growth of marigolds, the scientists carried out the procedure described in step 1 to step 5.

step 1 Prepare five containers, each with five marigold plants grown from seed.

step 2 Add 20 cm^3 of distilled water to one of the containers. This is the control.

step 3 Add 20 cm^3 of the root extract concentrations to the other four containers, so that each container has a different concentration of root extract.

step 4 Repeat step 2 and step 3 each day for 60 days.

step 5 After 60 days, remove the marigold plants from the soil and untangle the roots. Measure the length of the longest root for each plant.

The scientists replicated this procedure twice and calculated the mean maximum root length for the control and for the root extract concentrations.

The results are shown in Table 1.2.

Table 1.2

root extract concentration / mg cm^{-3}	**mean maximum root length after 60 days / mm**
control	163.0
2.5	153.2
5.0	156.4
7.5	152.0
10.0	141.6

(ii) Using the data in Table 1.2, calculate the percentage change in the mean maximum root length between the control and the roots treated with 10 mg cm^{-3} root extract concentration.

Show your working and record your answer to 3 significant figures.

percentage change ..... [3]

(e) A student suggested that this investigation could be improved.

With reference to the method used and the data in Table 1.2, state **and** explain **three** ways to improve confidence in the results.

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..... [3]

(f) To investigate the effect of the different root extract concentrations on the diversity of the microorganisms in soil, the scientists:

- took samples of soil from each of the containers
- identified and counted the number of species of microorganism present in the samples
- calculated the Simpson's index of diversity (*D*) values for the species of microorganism in each of the soil samples.

State **two** variables the scientists should have standardised when taking the soil samples.

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[Total: 19]

Question 2

Tumours may be described as benign or malignant. Malignant tumours can lead to greater complications for the person with the tumour.

Early identification of tumours, particularly malignant tumours, is important for effective treatment. Benign tumour cells and malignant tumour cells can look similar when viewed using a light microscope.

Some scientists wanted to develop a diagnostic test to identify tumour cells as benign or malignant. The scientists investigated whether the diameter of the cell nucleus could be used to identify the type of tumour cell as benign or malignant.

The scientists used a light microscope with a calibrated eyepiece graticule to measure the diameter of the nuclei of stained tumour cells.

Fig. 2.1 shows a photomicrograph of stained tumour cells viewed using a light microscope.

An eyepiece graticule was placed across the nucleus of one of the tumour cells.

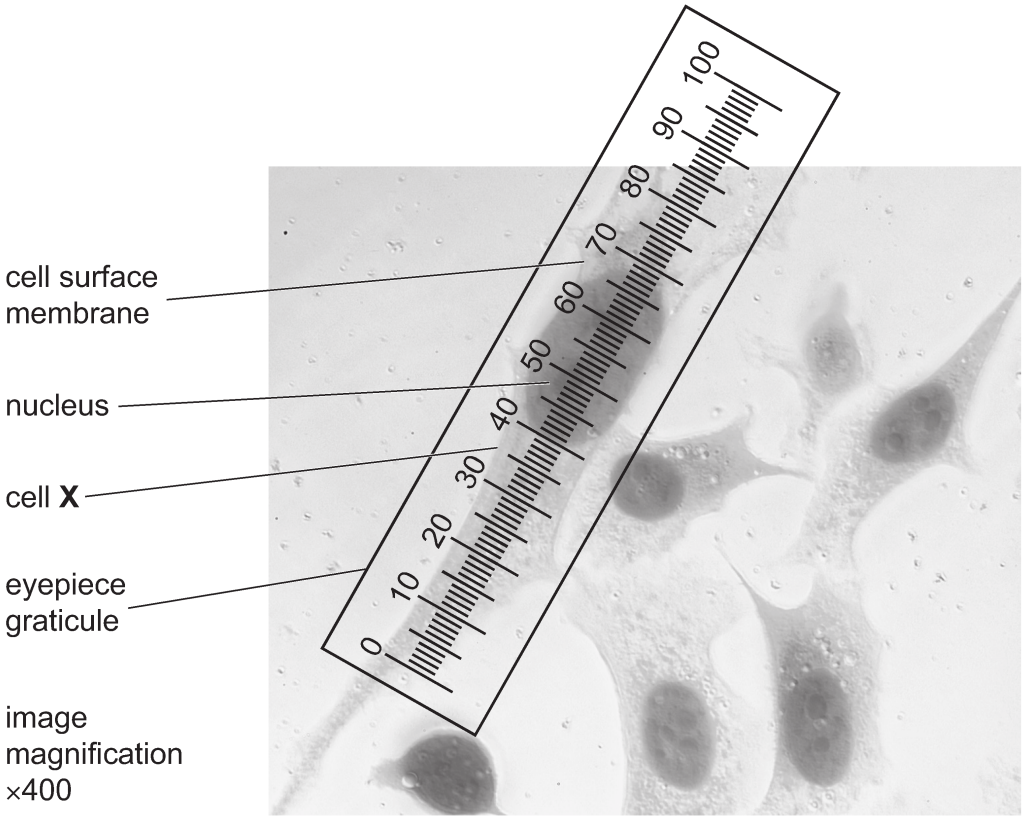

Fig. 2.1

The calibration of the eyepiece graticule scale is:

one eyepiece graticule division = 320 nm

(a) Use the calibration of the eyepiece graticule scale to calculate the actual diameter of the nucleus of cell **X**, shown in Fig. 2.1.

Show your working and state your answer in μm.

actual diameter of the nucleus of cell **X** = ... μm [3]

(b) For the investigation into whether the diameter of the nucleus could be used to identify the type of tumour cell, tumours of the thyroid gland (an endocrine gland) were used.

For the diagnostic test the scientists carried out a procedure using two different stains.

step 1 50 people diagnosed with benign tumours and 24 people diagnosed with malignant tumours were selected.

step 2 A sample of tumour cells was removed from the thyroid gland of each person.

step 3 The cells were stained with either Papanicolaou stain (Pap) or haematoxylin and eosin stain (H&E).

step 4 Samples were viewed using a light microscope with a magnification of ×400.

step 5 In each sample, the diameters of 100 nuclei were measured.

step 6 The measurements were made by one of the scientists who did not know the type of tumour cell they were measuring.

Table 2.1 shows the results of the investigation.

Table 2.1

category	mean nuclear diameter / μm	standard deviation / μm	standard error / μm
total benign cells (50 people) 5000 cells:	7.3	±0.8	±0.011
• Pap-stained cells (41 people) 4100 cells	7.3	±0.7	±0.011
• H&E-stained cells (9 people) 900 cells	7.2	±1.0	±0.033
total malignant cells (24 people) 2400 cells:	9.0	±0.6	±0.122
• Pap-stained cells (18 people) 1800 cells	9.0	±0.7	±0.016
• H&E-stained cells (6 people) 600 cells	8.8	±0.5	±0.020

(i) Suggest how the scientists can standardise the method of measuring the diameter of the 100 nuclei in step 5, so that valid comparisons can be made between benign and malignant tumour cells.

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..... [1]

(ii) Identify **one** variable that the scientists have standardised in step 1 to step 6, **other than** details of the method of measurement of the nuclei.

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(iii) The scientists used a *t*-test to compare the mean nuclear diameter of the Pap-stained cells and the H&E-stained cells in Table 2.1.

State a null hypothesis for the *t*-test.

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(iv) The *t*-value the scientists calculated had a probability (p) value greater than 0.25 ($p > 0.25$).

State **one** conclusion that can be made about the effect of using the two stains, Pap and H&E, on the mean nuclear diameter of the cells.

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(v) State **and** explain **one** conclusion that can be made from the data in Table 2.1 when comparing the results for the 5000 benign cells and the 2400 malignant cells.

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..... [2]

(vi) At the end of the investigation, the scientists evaluated their procedure and results. They identified some disadvantages of using the procedure as a diagnostic test.

Suggest **and** explain **two** disadvantages of this procedure as a diagnostic test.

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..... [2]

[Total: 11]

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Q1-Solution

(a) (i) Species of tree or type of tree.

(ii) 1. For each tree species, same number of plantations were selected.

2. Within each plantation same sized area of plot, i.e., $36\text{ m} \times 36\text{ m} = 1296\text{ m}^2$ was studied.

(b) A tape or line or string is used along with quadrats at certain positions along the tape or line to create belt transect. A starting point is selected for the transect or placement of tape. All transects used are of same length in plots of plantations. Quadrats of same size are used and sampling by quadrats is done at regular intervals by placing the quadrat at specific started distances along the tape or line or string used. Same method is used to identify each of the plant species in the quadrat, e.g., dichotomous key. Number of individuals of each plant species in each quadrat at each sampling point was counted and recorded. Percentage (%) cover of each plant species in each quadrat is estimated. In each plot, at least three different transects are used. In order to avoid risk of falling tress or their branches hitting the head, a hard hat is used. To avoid thorns, goggles, gloves and mask is used. Cover skin and use antihistamine medicines to avoid risks of irritants, allergy or infections.

(c) Data in Table 1.1, does not support as acacia and mango both have same means and have similar values of diversity index, *D*.

(d) (i)

concentration /mg cm^{-3}	volume of stock solution /cm^3	volume of (distilled) water /cm^3
7.5	37.5	12.5
2.5	12.5	37.5

(ii) $$\text{Percentage change} = \frac{163 - 141.6}{163} \times 100$$
$$= 13.129\% \approx 13.1\%$$

Answer Tips

(a) (ii) Same method or a belt transect was used in all plots of plantations. Sampling was done at the same time, i.e., same months in April, July and November (same seasons of the year).

(b) Animals or insects may bite or attack, so to avoid their infection, stings or injuries, trained professionals should be accompanied well equipped with first aid like antivenom, antihistamine, insect repellents, gloves, goggles, masks, skin covers etc. Correct footwear, walking sticks and watch where walking to avoid trip hazards in plantation terrains.

(c) Mango and invasive species have overlapping *D* values and means +/– SD, so hypothesis is not supported. However, it is partially supported as sal tree has the highest *D*-value and mean. So it has greater *D*-value and mean than both alien species.

(e) 1. More intermediate smaller values between intervals are used to have better idea of the trend or specific pattern.
2. Anomalous results are excluded to improve the reliability.
3. Repeat investigation at least twice with more marigolds, which gives better idea of trend and improves reliability.

(f) 1. Same mass of soil is taken as sample of soil from each of the containers.
2. Sample should be taken from same distance from roots or same position in the pot.

Q2-Solution

(a) Number of EPG divisions = 28

$$\text{One EPG division} = \frac{320}{1000}\text{nm} = 0.32\ \mu\text{m}$$

$$\text{Actual diameter of nucleus} = 28 \times 0.32 = 8.96\ \mu\text{m}$$

(b) (i) Measure the widest / longest part of each nucleus.

(ii) Measurements are taken at the same magnification i.e., ×400 and same number of nuclei, i.e., 100 are measured per person.

(iii) There is no significant difference between the mean nuclear diameters of the Pap-stained and H&E-stained cells.

(iv) Null hypothesis is accepted showing that there is no significant difference between nuclear diameter of cells stained by both stains.

(v) This technique can be used to distinguish between benign and malignant cells, as mean $\pm 2\,\text{SE}$ do not overlap. Mean nuclear diameter of benign cells is smaller than in malignant cells.

(vi) It is time consuming due to preparation, counting and measuring steps. There is shortage of skilled scientists using the technique correctly. It is also labour intensive and there is chance of human error due to small or very close values and large numbers counted.

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Answer Tips

(e) Repeat readings for 2.5 / 5.0 / 7.5 mg cm^{-3} to check the anomaly and improve reliability. Calculate standard error, standard deviation and confidence intervals to check for significance of differences between the means or for accuracy. Marigold clones are used to exclude genetic effects and improve validity. Measure the mass of all the roots to improve reliability. More precise measurements are taken by using Vernier calliper.

(f) Sample from same depth is taken. Sampling is also done at the same time.

Answer Tips

(b) (ii) Sample is taken from same tissue or thyroid gland and only same scientist measures all nuclei of cells. Type of tumour cell is not known to scientist measuring it.

(v) There is a significant difference between mean nuclear diameter of benign and malignant cells.

(vi) Procedure is painful, invasive and can cause infection or bleeding. Only one tissue, i.e., thyroid gland is used. Tissue damage might occur. Metastasis can also occur. Treatment may be delayed unnecessarily due to misdiagnosis. It is not feasible on large scale and may not be true of other tumours in other tissues.

Question 1

In the light-dependent stage of photosynthesis, electrons and hydrogen ions are accepted by the coenzyme NADP, which becomes reduced.

DCPIP is a dye that can act as an electron and hydrogen ion acceptor. The dye is blue when oxidised and colourless when reduced. In laboratory experiments, DCPIP can be used to follow the progress of the light-dependent stage because it can replace NADP as the acceptor molecule for electrons and hydrogen ions, as shown in Fig. 1.1.

oxidised DCPIP + electrons + hydrogen ions ⟶ reduced DCPIP
(blue) (colourless)

Fig. 1.1

The effects of various factors on the light-dependent stage of photosynthesis can be investigated by using suspensions of isolated chloroplasts (chloroplast suspensions) and DCPIP.

A student used DCPIP to investigate the effect of temperature on the rate of the light-dependent stage of photosynthesis in spinach, *Spinacia oleracea*.

The student prepared a leaf extract to make a stock chloroplast suspension and then carried out a preliminary experiment to determine a suitable concentration of chloroplast suspension to use in the investigation.

To carry out preliminary experiment, the student followed a set of instructions, steps 1 to 11.

1 Cut spinach leaves into small pieces and place these pieces in a blender containing ice-cold 10% sucrose solution buffered at pH 7.0.
2 Turn on the blender for 15 seconds and then filter the extract to remove all the small pieces of leaf.
3 Place the leaf extract in a centrifuge and spin at low speed.
4 Pour off the supernatant that contains the chloroplasts. Keep this **stock chloroplast suspension** ice cold and in the dark.
5 Prepare 5 different concentrations of the chloroplast suspension using 10% sucrose solution. The percentage concentrations are 10%, 20%, 30%, 40% and 50% of the stock chloroplast suspension.
6 Wrap 5 flat-bottomed tubes in black plastic film to prevent light entering.
7 Put 10 cm^3 of each concentration of stock chloroplast suspension into a flat-bottomed tube, and add 1 cm^3 of DCPIP solution to each tube. The chloroplast suspension is now blue-green in colour.
8 Place 1 of the tubes beneath a light source as shown in Fig. 1.2.

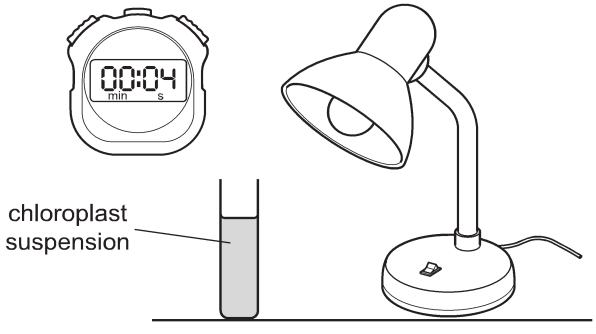

Fig. 1.2

9 Start a timer. Remove the black plastic film from the tube. Record the time taken for the DCPIP to decolourise so that the chloroplast suspension is green.

10 Calculate the rate of the light-dependent stage of photosynthesis by using the formula:

$$\text{rate} = \frac{1000}{t}$$

t = time taken in seconds for the chloroplast suspension to reach a green colour when all the DCPIP is decolourised.

11 Repeat step 8 to step 10 for the other tubes.

(a) Suggest **two** suitable control experiments that the student should carry out as part of the preliminary experiment.

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2. .....

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(b) The results of the preliminary experiment are shown in Table 1.1.

Table 1.1

percentage concentration of chloroplast suspension	**time taken for DCPIP to decolourise / s**	**rate of light-dependent stage of photosynthesis / s^{-1}**
10	351	.....
20	59	.....
30	21	.....
40	10	.....
50	5	.....

(i) Complete Table 1.1 by calculating the rate of the light-dependent stage of photosynthesis for each concentration of chloroplast suspension.

Give your answers to **one** decimal place. [2]

(ii) Plot a line graph of the data in Table 1.1 on the grid in Fig. 1.3 to show the effect of changing the concentration of the chloroplast suspension on the rate of the light-dependent stage of photosynthesis.

Fig. 1.3

[3]

(c) The student initially trialled the experiment using ice-cold distilled water instead of ice-cold 10% sucrose to make the stock chloroplast suspension in step 1. The DCPIP did **not** decolourise.

Suggest why the procedure worked when 10% sucrose solution was used but did **not** work when distilled water was used in step 1.

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(d) The preliminary experiment was carried out at 17 °C.

To investigate the effect of temperature on the rate of the light-dependent stage of photosynthesis, the student added some details to the instructions in steps 7, 8 and 9 to make sure that accurate results were recorded.

Describe an investigation that the student could follow to determine the effect of temperature on the rate of the light-dependent stage of photosynthesis.

- Use the results shown in Table 1.1 to decide on a suitable chloroplast suspension for the investigation.
- Include details of how you would take accurate results.
- Do **not** include a risk assessment.

Your method should be set out in a logical order and be detailed enough to allow another person to follow it.

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[Total: 15]

Question 2

The two-spot ladybird, *Adalia bipunctata*, is a species of small, flying beetle that is found in northern Europe and other parts of the world. The wings of these beetles are covered by two tough structures known as elytra, as shown in Fig. 2.1.

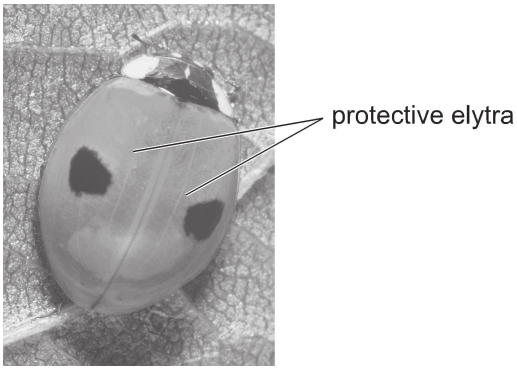

Fig. 2.1

A. bipunctata is an important predator of insect pests such as aphids. These insect pests feed on plants, including many crop species. Population numbers of *A. bipunctata* fluctuate in response to changes in the population numbers of their prey.

The elytra of *A. bipunctata* show phenotypic variation. The three most common morphs (forms) found in northern Europe are known as typica (**T**), quadrimaculata (**Q**) and sexpustulata (**S**).

- The typica morph, shown in Fig. 2.1, is mostly red with two black spots and is described as **non-melanic**.
- The other two morphs are described as **melanic** as they are mainly black with some red.
- The distribution of the colours in these three morphs is shown in Fig. 2.2.

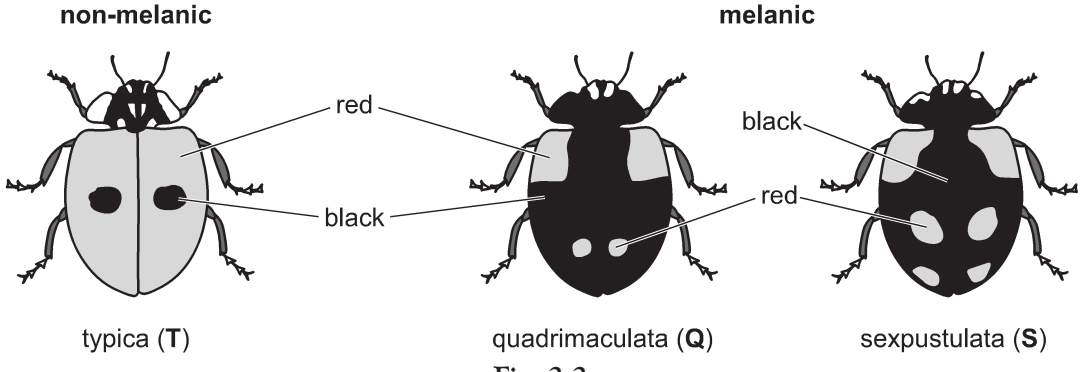

Fig. 2.2

In northern Europe, the populations of *A. bipunctata* hibernate (are inactive) during the winter. As the temperature increases in early spring, the populations become active. The populations remain active and produce three generations before the next winter begins.

(a) It was observed that in some areas in populations of *A. bipunctata*, the proportion of non-melanic to melanic phenotypes in the early spring was different to the proportion later in the year in the autumn.

Researchers carried out a study in one area of Germany to compare the proportion of non-melanic to melanic phenotypes in early spring with the proportion in autumn after three generations had been produced. The data were collected over a period of 12 years.

The results are shown in Fig. 2.3.

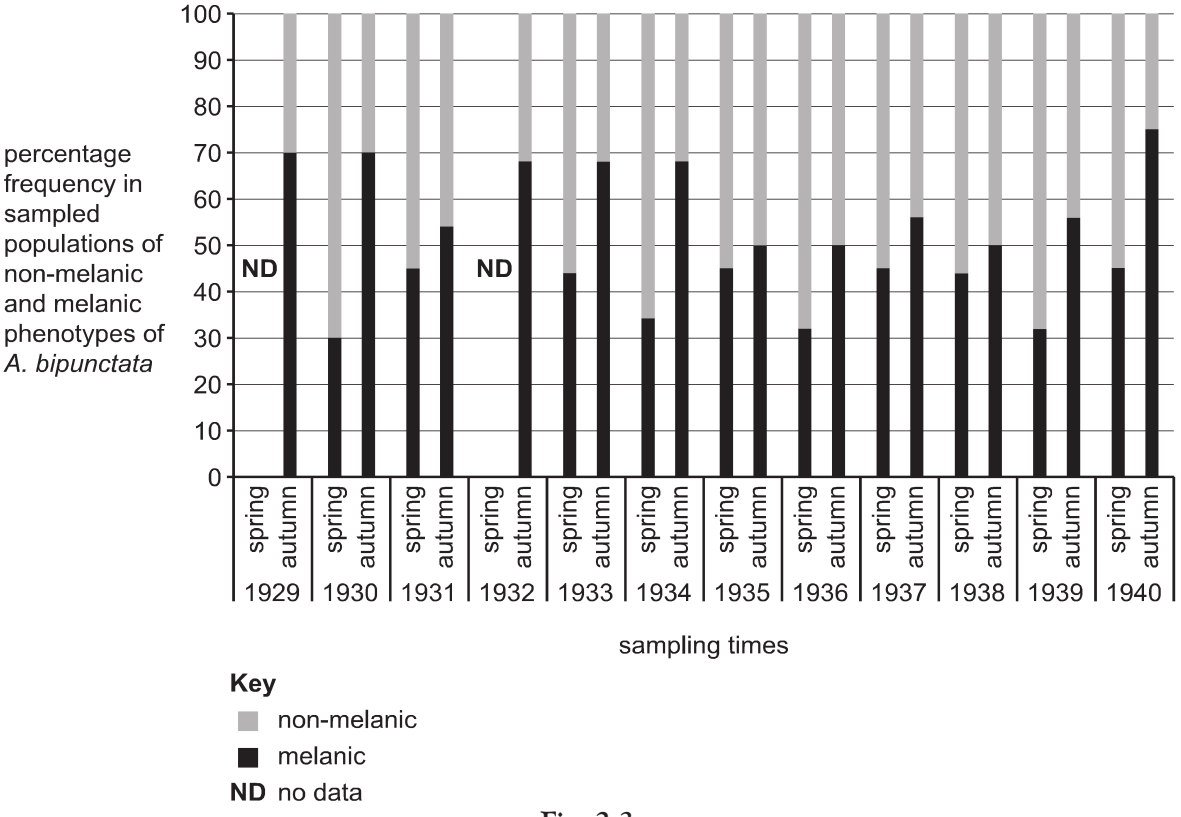

Fig. 2.3

(i) Outline a method that could be followed to collect the data shown in Fig. 2.3.

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..... [3]

(ii) The researchers concluded that there was a change in the proportion of the two phenotypes between spring and autumn, with the non-melanic phenotypes always showing a higher percentage frequency in the spring.

Suggest **two** pieces of extra information about the investigation that researchers should provide to improve confidence in their conclusion.

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(b) Environmental temperature is one of many different selection pressures that could be acting on populations of *A. bipunctata*. The melanic phenotypes, morphs **Q** and **S**, are considered to be better adapted to the climate in northern Europe as they absorb heat energy more readily than the non-melanic phenotype, morph **T**.

Scientists investigated the effect of temperature on the body temperature of the two different phenotypes.

- The four individuals used in the investigation were collected while they were hibernating (inactive) and were kept outdoors for six weeks before the investigation.
- The individuals were weighed and arranged into pairs, each with approximately the same mass.
- A temperature sensor was fixed to the lower (ventral) surface of each individual.
- The temperature of each individual was recorded for 9 minutes. During that time, a heat-emitting lamp positioned directly above the individuals was switched on at 30 s and switched off at 5 minutes.

The results are shown in Fig. 2.4.

(i) The researchers concluded that melanic morphs warm up more quickly than non-melanic morphs.

Explain how the information shown in Fig. 2.4 supports this conclusion.

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(ii) State **two** further conclusions that can be made from the results shown in Fig. 2.4.

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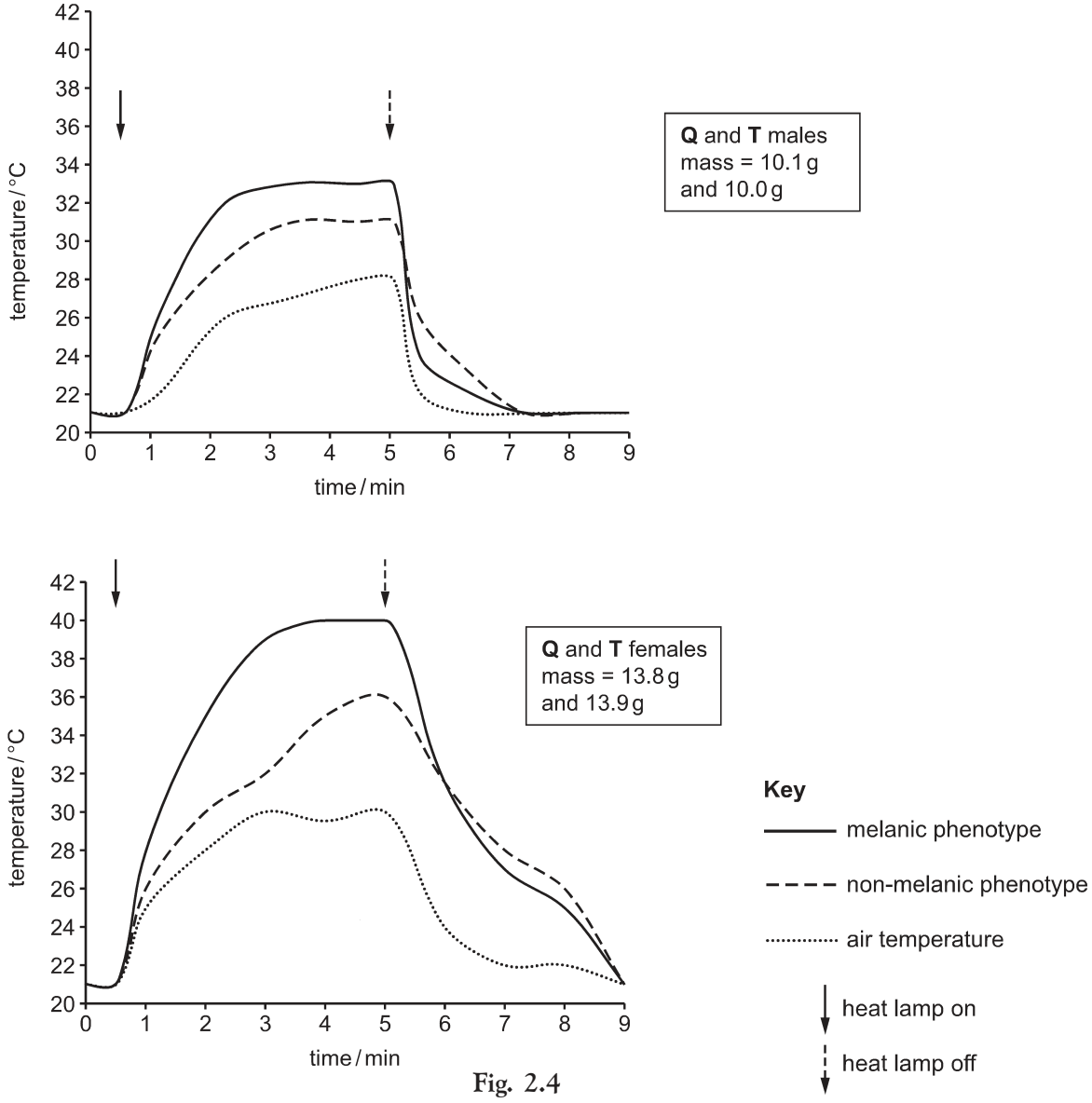

Fig. 2.4

(c) The scientists noticed that there appeared to be a relationship between body mass and the increase in body temperature. The scientists therefore continued to take results from another 17 individuals. They recorded the maximum difference between body temperature and the air temperature for each individual in their investigation.

All the results of the investigation are shown in Table 2.1 and Fig. 2.5.

Table 2.1

phenotype	morph	sex	mass / g	maximum temperature difference / °C
non-melanic	T	female	6.5	0.0
		male	8.4	1.1
		male	9.2	2.7
		male	10.0	2.9
		male	10.8	4.0
		female	11.5	5.4
		female	12.0	5.9
		female	13.1	5.3
		female	13.9	7.3
melanic	Q	male	8.4	3.5
		female	8.6	3.7
		male	10.1	5.3
		female	10.4	4.8
		female	11.0	8.5
		male	11.7	7.5
		female	13.1	7.3
		female	13.8	10.2
	S	male	8.6	4.1
		male	9.1	3.7
		male	10.8	5.9
		female	13.6	10.0

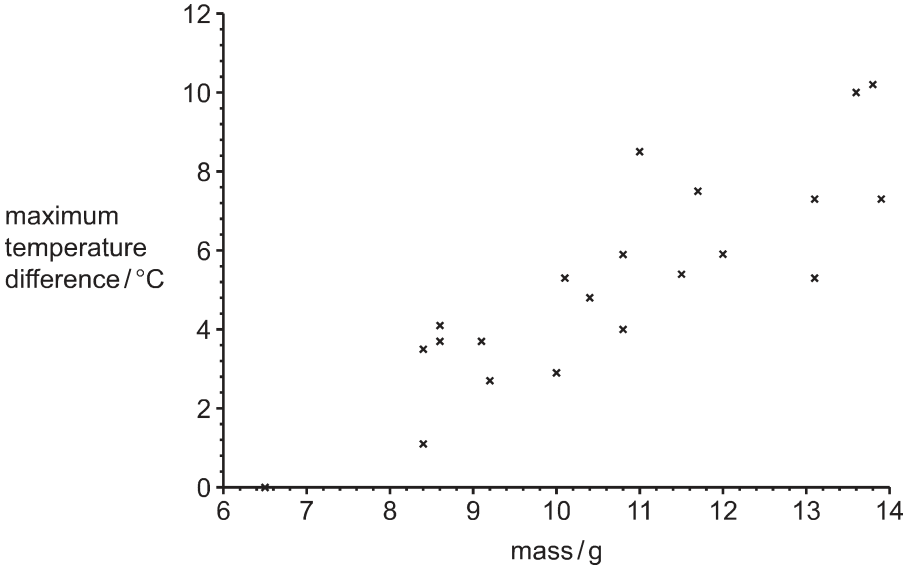

Fig. 2.5

State a statistical test that could be used to analyse the relationship shown in Table 2.1 and Fig. 2.5. Justify your choice.

statistical test .....

justification .....

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[3]

(d) The researchers assumed that the appearance of the elytra was controlled by a single gene with two alleles. Breeding experiments showed that the melanic phenotype is dominant to the non-melanic phenotype.

Researchers made the hypothesis:

Some melanics are homozygous dominant, and some melanics are heterozygous.

Outline a breeding experiment that could be carried out to test this hypothesis, and state the results you would expect if the hypothesis is supported.

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..... [3]

[Total: 15]

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Q1-Solution

(a) 1. Chloroplast suspension can be replaced with distilled water or boiled chloroplast suspension may be used.

2. Chloroplast suspension plus DCPIP may be kept in the dark.

Answer Tips

(a) DCPIP can be replaced with distilled water.

(b) (i)

percentage concentration of chloroplast suspension	time taken for DCPIP to decolourise / s	rate of light-dependent stage of photosynthesis / s^{-1}
10	351	2.8
20	59	16.9
30	21	47.6
40	10	100.0
50	5	200.0

(ii) Refer to graph on next page.

(c) Procedure worked with 10% sucrose solution because sucrose solution has same water potential as in the chloroplast. So water will not enter the chloroplast by osmosis, as there is no water potential gradient. Chloroplasts will not burst.

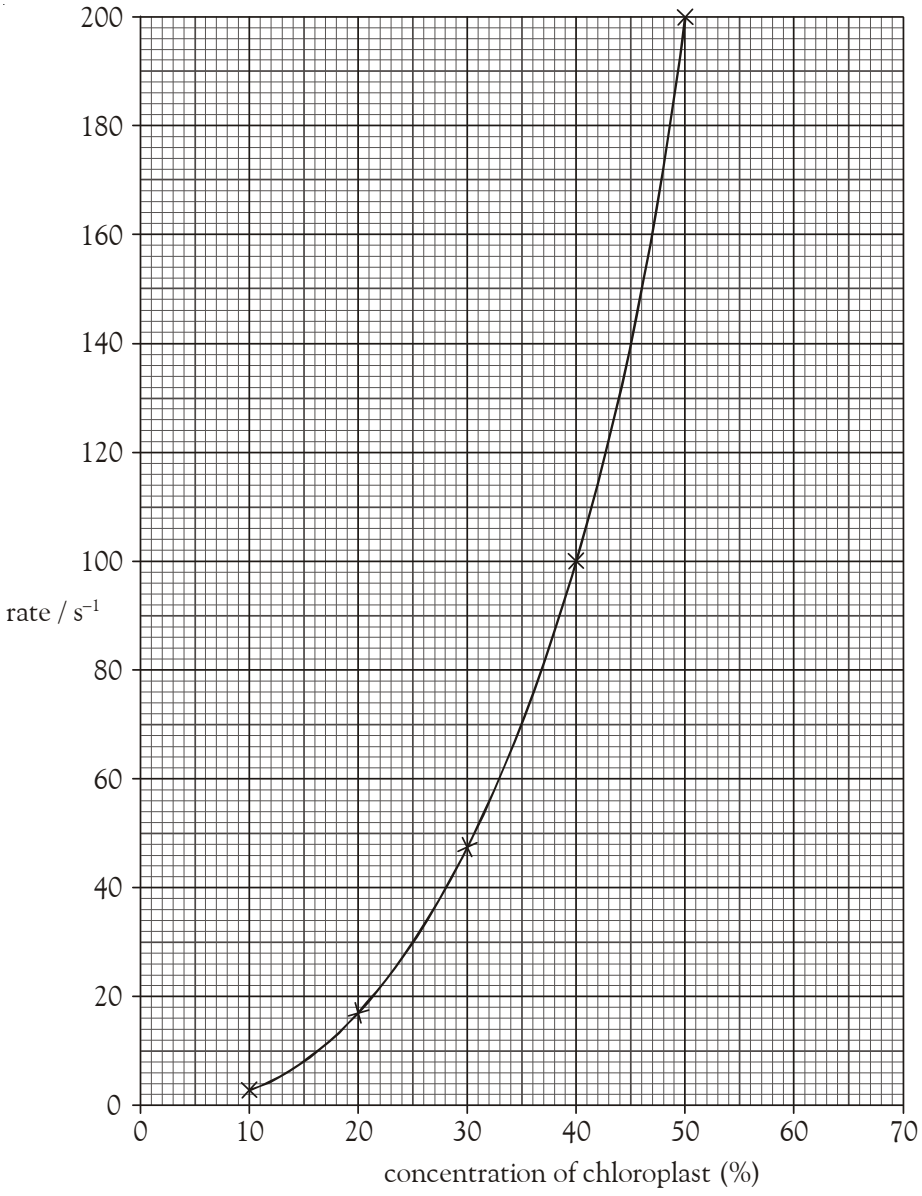
(d) For this investigation, 50% or 40% suspension of chloroplast can be used because in both suspensions, rate of photosynthesis is quite fast. Experiment can be performed in different temperatures like 10, 20, 30, 40, 50 & 60 °C. A water bath is set up for each temperature to keep it constant. Same volume of chloroplast suspension is poured in each test tube and all test tubes are kept in water baths for same time to reach at set temperature before exposing to light. Swirl chloroplast suspension before exposing to light. Light source with same wattage of bulb is used and bench lamp is placed at fixed distance from test tubes to ensure the same light intensity. Experiment is performed in a dark room. In one test tube sample of suspension without DCPIP is kept as a green colour standard to match the decolorisation of DCPIP at different temperatures. Experiment is repeated at each temperature and mean is calculated.

Answer Tips

(c) By using only water without sucrose, water enters chloroplast by osmosis due to water potential gradient and chloroplast bursts, so light reactions do not occur.

(d) Thermostatically stable constant temperature of water bath is used for accuracy. Experiment is repeated for reliability of results. Dark room is used to avoid light entering from the surroundings.

(ii)



Answer Tips

(b) (i) Rates are calculated by $\frac{1000}{t}$ for each concentration and rounded to one decimal place.

Q2-Solution

(a) (i) Random or systematic sampling can be done by using quadrats. Number of each phenotypes is counted in quadrats. Ladybirds can be captured from each quadrat and mean is calculated. Alternatively number of each phenotypes is marked and released to use mark-release recapture method for estimation of their population.

(ii) Data for spring 1929 and spring 1932 is required. Sample size is increased and multiple sampling sites in same area of Germany should be included. Statistical analysis is also needed.

Answer Tips

(a) (i) Ladybirds are marked by suitable marking technique which is not harmful but marks are not removable. Few days after their release, second sample is captured and number of each phenotypes is counted. Number of marked are also counted. Then their population is estimated as,

$$\frac{\text{No. in 1st sample} \times \text{No. in 2nd sample}}{\text{No. marked in 2nd sample}}$$

(b) (i) Line for the melanic phenotype is quite steeper compared to non-melanic phenotype. It shows that melanic ladybirds absorb heat quicker and warm up.

(ii) All ladybirds have the same starting temperature and all warm up when lamp is on. After lamp is switched off, all ladybirds return to their original or starting temperature. Melanic ladybirds reach higher temperature and also cool down faster.

(c) Statistical test: Pearson's linear correlation.

Justification: Data for temperature and mass is paired or linked. Data is also continuous. Scatter diagram suggests a linear relationship or correlation. There are at least five paired observations enough to apply linear correlation test.

(d) Melanic ladybirds are crossed with non-melanic. Homozygous dominant melanic parents will only have melanic offsprings and heterozygous melanic parents will have both phenotypes in offsprings. Many breeding pairs are used in experiment. Each breeding pair is kept separate from other pairs. Different phenotypes of offsprings of each pair are counted.

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Answer Tips

(b) (ii) Females warm up quicker and also reach higher temperature than males. Females take longer to cool down. Heavier ones warm up quicker and also reach higher temperature than lighter ones. Heavier ladybirds take longer to cool down. The temperature plateaus for all except non-melanic females.

(c) Alternatively spearman's rank correlation is also applied if data is paired or linked. Data is ordinal or in intervals. Scatter diagram shows increasing correlation and there are more than five paired observations.

(d) Offsprings of each breeding pair are kept separate and counted.