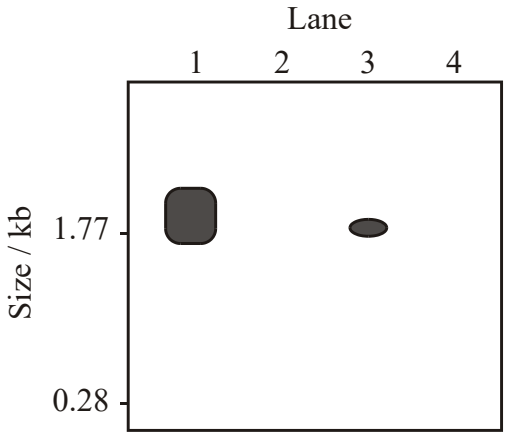
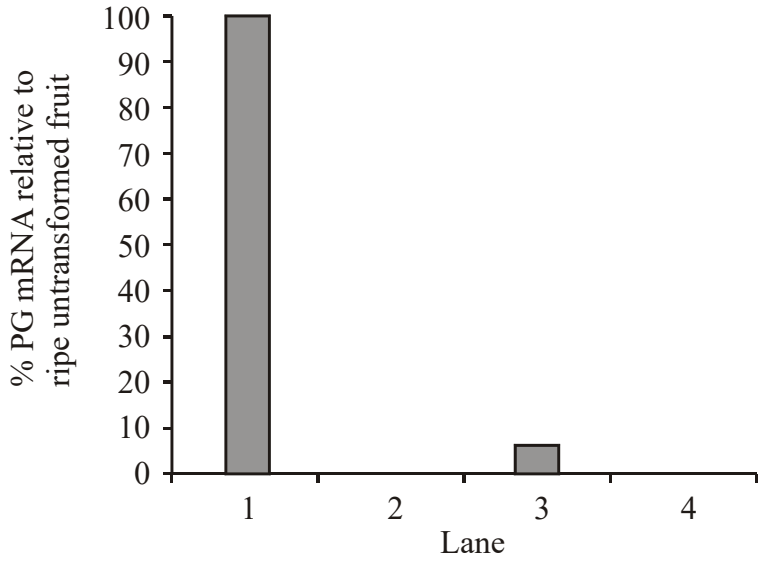
Antisense RNA (6 marks)

Polygalacturonase (PG) plays an important role in fruit softening by making the pectin of the cell wall more soluble. It is synthesized only when the fruit is ripe. In order to slow down the ripening of tomatoes (*Lycopersicon esculentum*), antisense RNA technology was used. Messenger RNA from untransformed and transformed fruit was hybridized to a radioactively labelled probe specific to the PG sense strand. The results of a gel electrophoresis of mRNA are given below. (The size of the mRNA strands is expressed in kilobases, kb.) The histogram shows these results expressed as the percentage of PG mRNA in ripe untransformed fruit.





Lane 1: Ripe untransformed fruit

Lane 2: Unripe untransformed fruit

Lane 3: Ripe transformed fruit

Lane 4: Unripe transformed fruit

a. State the percentage of PG mRNA in ripe transformed fruit. *(1 mark)*

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b. Compare the results obtained for ripe and unripe fruit. *(2 marks)*

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c. Using the information provided, explain how the antisense technology affects transformed fruit. *(3 marks)*

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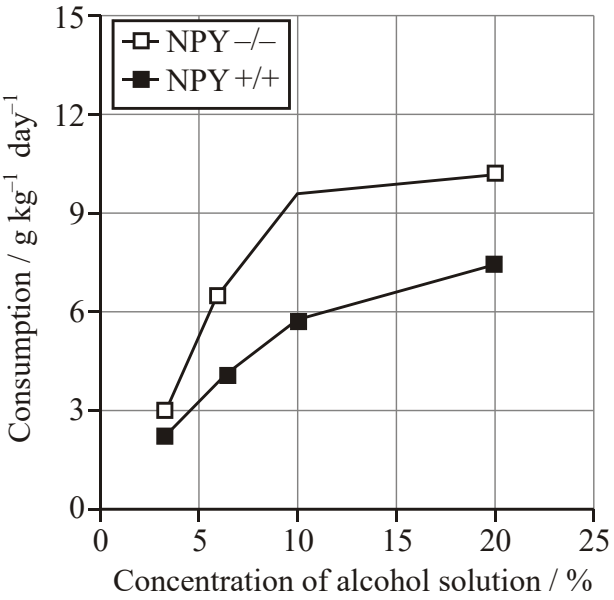
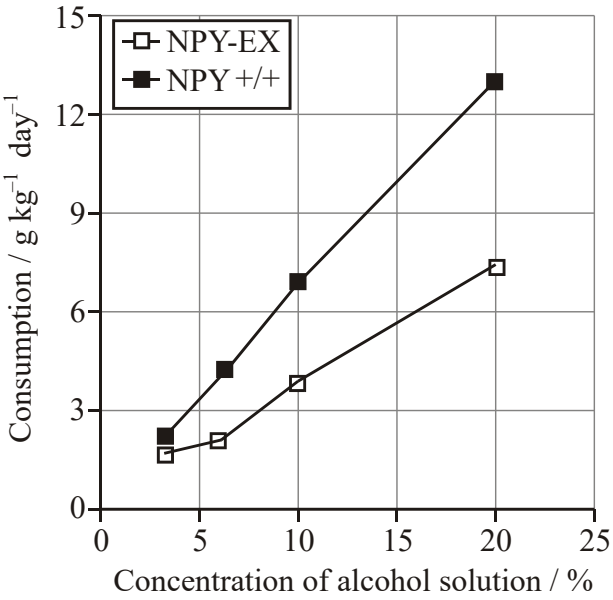
Alcohol Consumption (13 marks)

Rats were bred for several generations to prefer alcohol (ethanol) consumption. When tested, it was discovered that the brains of these rats possessed lower quantities of the chemical neuropeptide Y (NPY). To test the hypothesis that lower quantities of NPY leads to a preference for alcohol, rats were genetically engineered to be NPY deficient (genotype NPY –/–), or to produce an excess of NPY (NPY-EX). In separate experiments, the two groups were compared to normal rats (in terms of their alcohol preference) possessing the genotype NPY +/+. The groups were offered solutions of increasing alcohol concentration. The quantity of each solution consumed per day was measured.





a. Calculate the difference in consumption of the 6% alcohol solution in figures 1 and 2. *(2 marks)*

i. NPY –/– and NPY +/+ rats (figure 1) .....

ii. NPY-EX and NPY+/+ rats (figure 2) .....

b. Compare the alcohol consumption of the NPY –/– rats with the NPY-EX rats. *(3 marks)*

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c. Identify the relationship between NPY levels and alcohol consumption. *(1 mark)*

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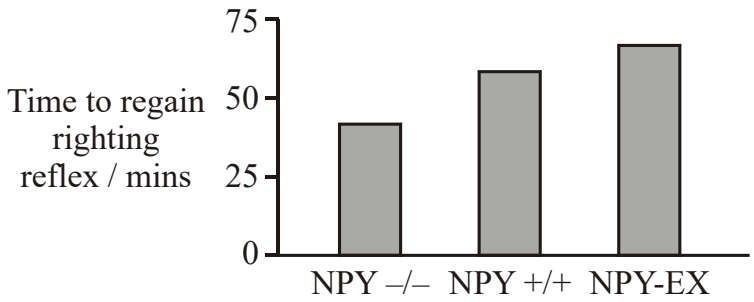
An experiment was carried out to test the hypothesis that an increase in preference for alcohol might be related to a decrease in sensitivity to its effects. Rats were injected with a sample of alcohol and then assessed for the length of time it took for them to regain the righting reflex. (The righting reflex refers to the ability of the rat to return to its feet after being placed on its back.)



d. Deduce the relationship between NPY levels and the time required to regain the righting reflex. *(3 marks)*

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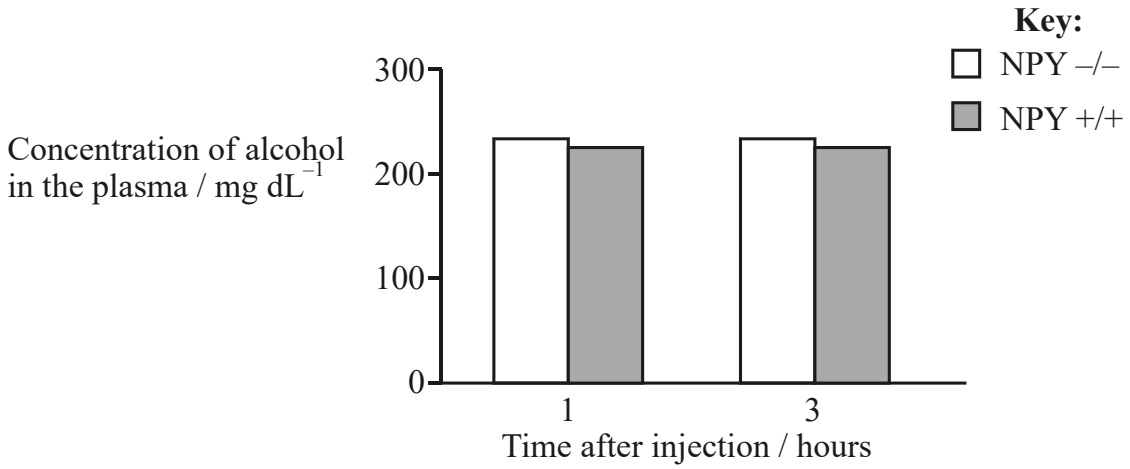
An additional experiment was carried out to determine whether differences in sensitivity to the effects of alcohol might be related to differences in the rats' ability to remove alcohol from their blood. Rats were injected with alcohol and blood samples were taken one hour and three hours later to determine alcohol levels. The results are shown below.



e. Evaluate the hypothesis that differences in sensitivity to the effects of alcohol might be related to differences in the ability of the rats to remove alcohol from their blood. *(2 marks)*

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f. Using all the data, outline the relationship between preference for alcohol and sensitivity to the effects of alcohol. *(2 marks)*

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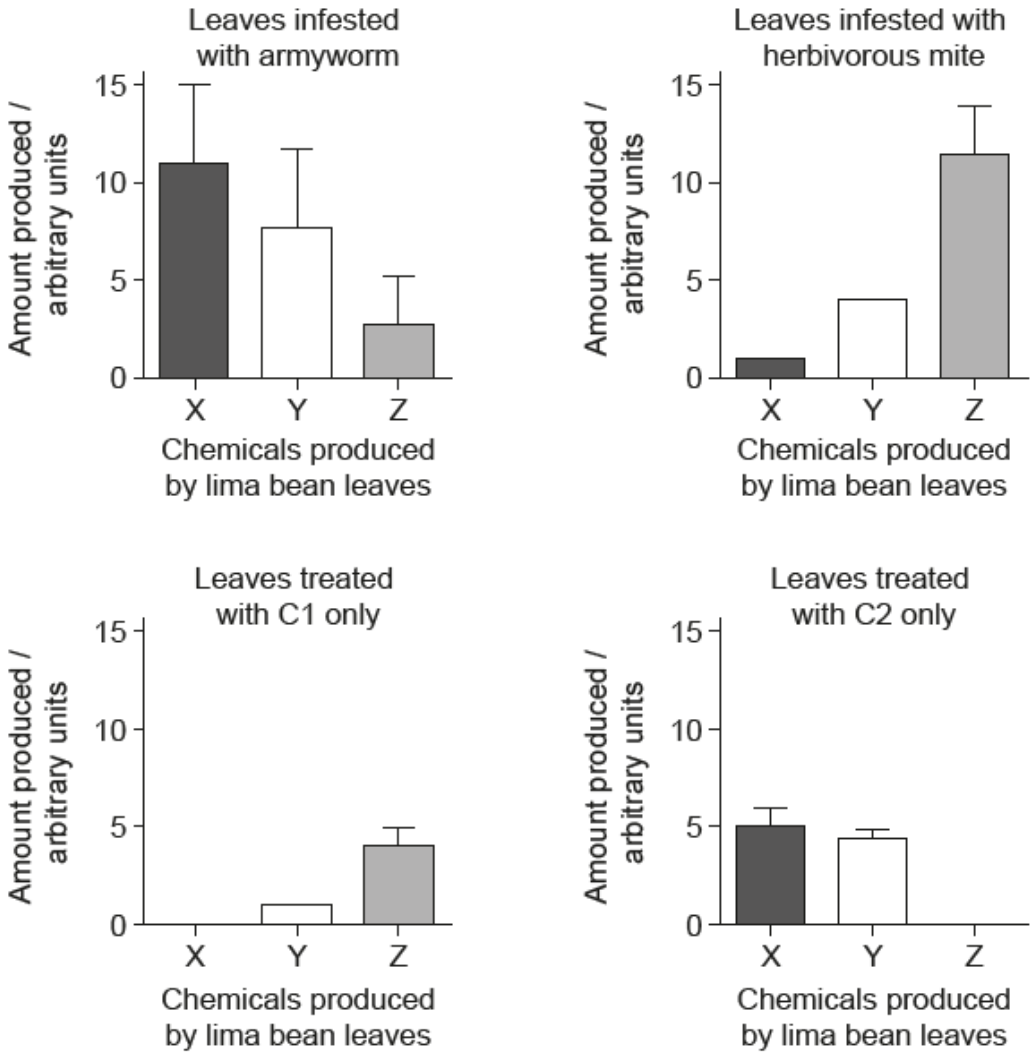
Alcohol Consumption (13 marks)

Organisms often release chemicals when attacked as part of their defence system. Scientists studied lima bean plants (*Phaseolus lunatus*) infested with either armyworms, *Spodoptera exigua*, or herbivorous mites, *Tetranychus urticae*. Both organisms feed on the leaves, causing the leaves to release chemicals.

The study was conducted to see which defence chemicals were produced by lima bean leaves when infested by armyworms or herbivorous mites. The scientists identified a mixture of compounds (C) released by the plant when attacked. Two of the chemicals in this mixture were identified (C1 and C2). The scientists hypothesized that the defence chemicals in C act as signals to produce other chemicals (X, Y and Z) that are also involved in the defence of the plant.

The graphs show the amounts of chemicals X, Y and Z produced when the plants were infested by either one of the two herbivores or treated with the different chemicals C1 or C2.



a. Distinguish between the data for the leaves infested with the armyworm and the leaves infested with the herbivorous mite. *(3 marks)*

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b. Compare and contrast the effects of treatment of the leaves using C1 and C2 with the effects of infestation. *(3 marks)*

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RNA was collected from leaves of the plants after each treatment (armyworm, herbivorous mite and the chemicals C1 and C2). DNA copies of the extracted RNA were made by a process called reverse transcription. Targeted genes in the DNA were then amplified.

c. Identify the process that was used to amplify the targeted genes. *(1 mark)*

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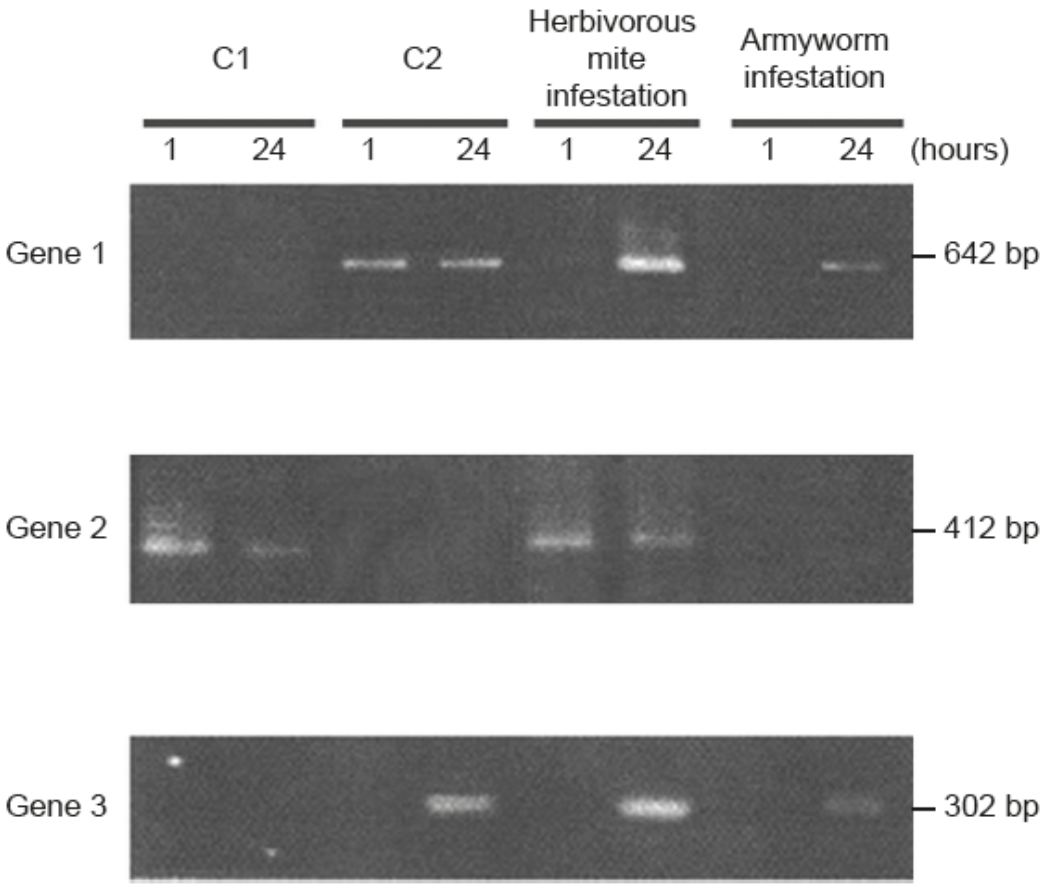
The scientists then used the transcribed DNA of each treatment to study the activation of three genes of the plants' defence system. The DNA was separated by gel electrophoresis. The activation was tested one hour after treatment and again after 24 hours.



d. Deduce, with a reason, which gene is first transcribed when exposed to C2. *(1 mark)*

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e. Each gene is activated by one or more of the treatments. Using the data shown above, discuss the impact of the herbivorous mite infestation on gene activation compared to treatment with C1 and C2. *(3 marks)*

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f. Using the gene activation data, deduce, giving **two** reasons, whether the armyworm or the herbivorous mite has infested lima bean plants over a longer period of time. *(2 marks)*

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Answers

1a *(1 mark)*
6 (± 2) (%)

1b *(2 marks)*
a. only ripe transcribe PG mRNA / ripe tomatoes produce more PG mRNA than unripe
b. band at 1.77 kb only in ripe

1c *(3 marks)*
a. no effect on unripe fruit
b. band at 1.77 kb much smaller in transformed / less PG mRNA produced in transformed ripe fruit
c. antisense mRNA combines with sense mRNA
d. inactivating the translation / less translation
e. less PG to solubilize pectin of wall
f. fruit takes longer to ripen

2a *(2 marks)*
a. 2.8 (±0.5) g kg^{-1} day^{-1}
b. 2.0 (± 0.5) g kg^{-1} day^{-1}
*Award **[1 max]** if units are not included or wrong units used. It is not necessary to indicate if the difference is positive or negative.*

2b *(3 marks)*
a. NPY – / – consumes more alcohol than NPY-EX (at all concentrations)
b. consumption increases at a (relatively) constant rate (above 6%) with concentration for NPY-EX, but levels off at higher concentrations for NPY – / –
c. as alcohol concentration increases both NPY – / – and NPY-EX rats consume more
d. NPY – / – consumes more alcohol than NPY + / + and NPY-EX consumes less than NPY + / +
e. biggest difference from NPY + / + for NPY-EX is at 20%, but for NPY – / – it is at 10%
Accept use of word control.

2c *(1 mark)*
NPY levels are inversely related to alcohol consumption / the lower the NPY levels, the more alcohol consumption
Accept the converse.

2d *(3 marks)*
a. NPY – / – takes least time to regain the reflex
b. NPY-EX takes most time to regain the reflex
c. NPY-EX is the most sensitive to effects of alcohol / NPY – / – is the least sensitive to effects of alcohol
d. the higher the NPY levels the more time taken to regain the reflex
e. NPY– / – / under-expression has more of an effect than NPY-EX / over-expression

2e *(2 marks)*
a. there is no difference / small difference in blood levels between groups at 1 hour and 3 hours / decrease from 1 to 3 hours is the same for both
b. therefore, the hypothesis does not appear to be justified

2f *(2 marks)*
a. NPY-EX does not prefer alcohol and is sensitive to effects of alcohol / NPY – / – prefers alcohol and is not sensitive to effects of alcohol
b. therefore, alcohol preference is inversely related to sensitivity to effects of alcohol / the less sensitive the rats are to alcohol, the more they consume it

3a *(3 marks)*

a. armyworm «infestation» produced more X than Y than Z/decreasing amounts ***AND*** herbivorous mite showed the opposite pattern/more Z than Y than X
b. armyworm «infestation» produced more X than herbivorous mite
c. armyworm «infestation» produced more Y than herbivorous mite / Y is the middle value for both. *(Accept OWTTE)*
d. armyworm «infestation» produced less Z than the herbivorous mite
e. other valid distinction

Clear distinction required not simple lists of values. For mp b-d accept vice versa.

3b *(3 marks)*

a. C1 caused the leaf to produce two of the same chemicals/Y and Z as the attack of herbivorous mites in a similar pattern «but in lower quantities» *(OWTTE)*
b. C1 produces the least «total» amount of chemicals of all the treatments
c. C2 has very similar pattern to those caused by the armyworms «but in lower quantities» *(OWTTE)*
d. **both** herbivores caused a greater production of chemicals/all three chemicals compared to either C1 or C2
e. armyworms cause the greatest total amount of chemical production of any of the other treatments
f. other valid comparison of chemical effect versus herbivore effect

Clear comparison required between herbivore infestation and chemical treatment not simple lists of values.

3c *(1 mark)*

PCR. *(Accept RT-PCR)*

3d *(1 mark)*

gene 1 is first transcribed «after C2 treatment» as it shows activation after one hour

3e *(3 marks)*

a. herbivorous mites induce activation of gene 2 first «at 1 hour» **and** then also gene 1 and gene 3 «at 24 hours» ***OR*** herbivorous mite «infestation» is the only treatment to affect all three genes/leads to greater gene expression overall
b. gene 2 activation similar for mite and C1 «at both 1 and 24 hours»
c. gene 3 activation similar for mite and C2 «both at 24 hours»
d. gene 1 activation slower for mite compared to C2 **but** more intense (than C2 at 24 hrs). *(Both parts OWTTE required for mpd)*
e. gene 1 and gene 3 expressed in higher amounts «after 24 hours» in mite infestation compared to C2

3f *(2 marks)*

a. the greater «gene expression» response of the lima bean plant to the mite infestation indicates a longer evolutionary relationship. *(OWTTE)*
b. herbivorous mites cause more genes to be expressed/higher intensity of gene activation
c. herbivorous mites cause a more immediate/earlier response in gene activation