4.1.1. Laboratory I - Animal Systematics and Morphology

Length of the practical test – 60 minutes; 40 points
Tools and Equipments
Microscope, microscopic slides, cover slips, preparation needle, forceps, 10 Petri Dishes, labelled **A** to **J**, permanent marker. There are specimens of one species in each vial.

Introduction
Scientists have investigated the fauna of a freshwater lake. Samples of benthos, plankton and surface dwelling animals were taken to characterise the fauna of aquatic animals and relationships among them. The samples were sorted, then preserved in formaldehyde and finally transferred to a 70% solution of ethyl alcohol, or stored alive.

Tasks
Q. 1. Fill in the answer code of the phylum for the specimens in each vial in the answer sheet.
The jury will check only the answers in the answer sheet! ***(5 points)***

Answer codes:
01 Arthropoda
02 Annelida
03 Porifera
04 Platyhelminthes
05 Mollusca

Vial	A	B	C	D	E	F	G	H	I	J
Phylum										

Q. 2. Fill in the answer code of the taxonomic units for specimens in each vial in the answer sheet. ***(5 points)***

Vial	A	B	C	D	E	F	G	H	I	J
Taxon										

Answer codes:
01 Crustacea
02 Diptera
03 Heteroptera
04 Hirudinea
05 Turbellaria
06 Odonata
07 Coleoptera
08 Lamellibranchiata
09 Euspongia

Q. 3. Mark with crosses in the table in the answer sheet the observed characters for the species for specimens in each vial.

Characteristics	**Vials**									
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
K. Laterally flattened body										
L. Abdomen covered by elytrae										
M. Body naked										
N. Labium with hooks										
O. Piercing-sucking mouth parts										
P. Swimming bristles on body										
R. Eyes absent										
S. Eyes rudimentary										
T. Eyes well developed										

(10 points)

Q. 4 A. Prepare 2 whole-mount microscope slides (I and II) for two specimens with the characteristics mentioned below. Use the provided materials. Mount the specimens in glycerine. You will get 1 point for choosing the correct specimen and 1 point for a well-prepared slide.
Please raise your hand when you have prepared both slides!
Slide I
Specimen with clearly visible head capsule, spiracles for breathing of atmospheric air and swimming bristles on the body.
Slide II
Specimen with antenna and antennula, laterally flattened body and compound eyes, planktonic.

(4 points)

Q. 4 B. Select the common characteristic for both animals and write the answer code in the space provided .

Answer codes:
01 Free swimming **02** Attached to plants **03** Benthic

(1 point)

Q. 5. Fill in the answer sheet with the appropriate code of gas exchange for the specimens in each vial.

Vial	A	B	C	D	E	F	G	H	I	J

Answer codes:
01 Spiracles and tracheae **02** Surface of body **03** Gills or rectal gills

Codes										

(5 points)

Q. 6. Based on each pair of characteristics provided, complete the diagrammatic dichotomous key in the answer list by filling in the letters of specimens (A-J) in the appropriate empty spaces in the boxes.

(10 points)

Answer codes:

01 Legs present
02 No legs

03 Six legs
04 More than six legs

05 Planktonic animal
06 Benthic animal

07 Adult, no gills
08 Larva, rectal gills present

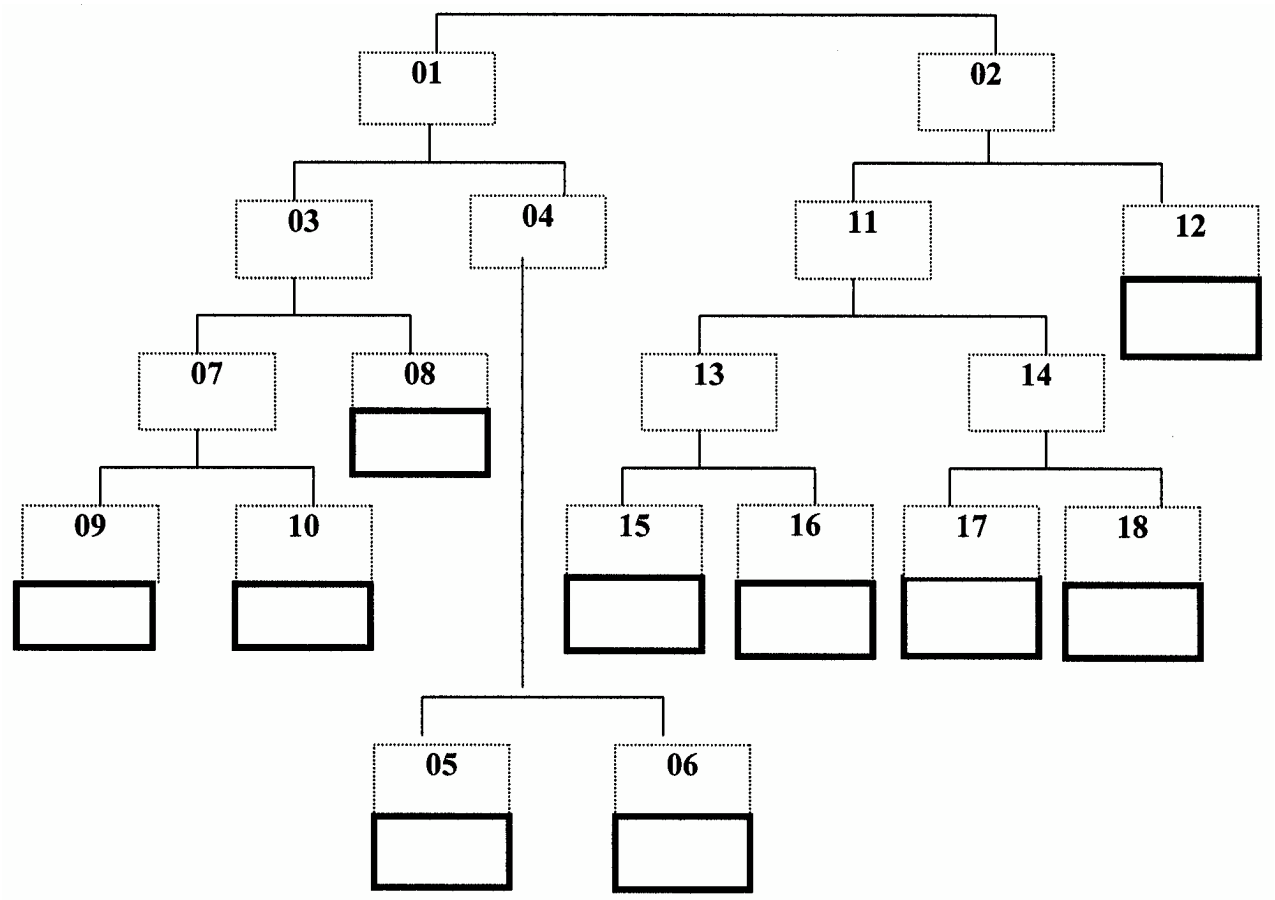
09 Swimming legs, live in the water
10 Running legs, live on the water surface

11 Definite shaped body
12 Indefinite shaped body

13 Body segmented
14 Body without segmentation

15 Head capsule developed
16 No developed head capsule

17 Body naked
18 Body covered by shell



4.1.2. Laboratory II - Plant Systematics, Anatomy and Physiology

Length of the practical test - 60 minutes; 40 points
In the laboratory you must solve 3 tasks

Task 1 – Plant Systematics
Task 2 – Plant Anatomy
Task 3 – Plant Physiology

Task 1 – Plant Systematics

Materials and instruments
You will use the instrument set that you received upon registering for the 13th IBO!
You will also use other instruments and materials: samples No. 1-8, magnifying glass
You have 8 plants (samples 1-8) which can belong to the following plant families (A-J)

In the code table:
Code Table

A. *Apiaceae*	E. *Fabaceae*	H. *Poaceae*
B. *Asteraceae*	F. *Geraniaceae*	I. *Ranunculaceae*
C. *Brassicaceae*	G. *Lamiaceae*	J. *Rosaceae*
D. *Araceae*		

By morphological characteristics, determine the respective families (from those given in the code table), for the samples (1-8)!

Q1. Identify each sample in the dichotomous identification key below. Enter the sample number in the provided windows in the answer sheet. ***(8 points)***
Write the family codes (A-J) in the windows provided beside the appropriate sample numbers! ***(8 points)***

(continued on next page)

Identification key

Thesis Nr.		Sample Nr. (1-8)	Family code (A-J)
1.	Flowers without perianth. Venation parallel … **2.**		
–	Flowers with calyx and corolla. Venation netted … **3.**		
2.	Inflorescence spike		
–	Inflorescence panicle as a dense cylinder		
3.	Inflorescence head		
–	Flowers single or in an inflorescence, that is not a head … **4.**		
4.	Flowers actinomorphic … **5.**		
–	Flowers zygomorphic … **7.**		
5.	Leaves entire or lobed… **6.**		
–	Leaves separated. Inflorescence of compound umbels. $G_{(2)}$		
6.	$Ca_5\ Co_5\ A_5+5G_{(5)}$		
–	$Ca_2+2Co_2+2A_2+4\ G_{(2)}$		
7.	Leaves opposite, entire; fruit – nutlet		
–	Leaves alternate, compound; fruit – legume		

Task 2 – Plant Anatomy

Materials and instruments:

You will use the instrument set that you received upon registering for the 13th IBO!
You will also use other instruments and materials: sample No. 9, pith of black elderberry (for fixing in task 2), microscope, stain mixture, Petri Dish with water, distilled water, microscope, slides and coverslips, razor blade, filter paper, cloth material.

Cut the pith of black elderberry *(Sambucus nigra)* lengthwise in half with a razor blade. Holding with hands, secure sample No. 9 lengthwize between the halves (Figure 1. A-D). The black elderberry pith is used only for fixing. Holding the black elderberry pith in one hand and the razor blade in the other, prepare cross-sections of sample No. 9 (Figure1E) and place them in the water in the Petri Dish! Choose the three best cross-sections (without the black elderberry pith) and place them on the microscope slide. Add a drop of Astra blue (stains cellulose) and safranin (stains lignin) mixture. After 0.5 minutes, remove the stain with the filterpaper, and add a drop of distilled water, and remove them with the filter paper. Twice repeat rinsing with water. Add a drop of water and place the coverslip over the cross-sections.
The quality of the cross-section and the preparation will be assessed!

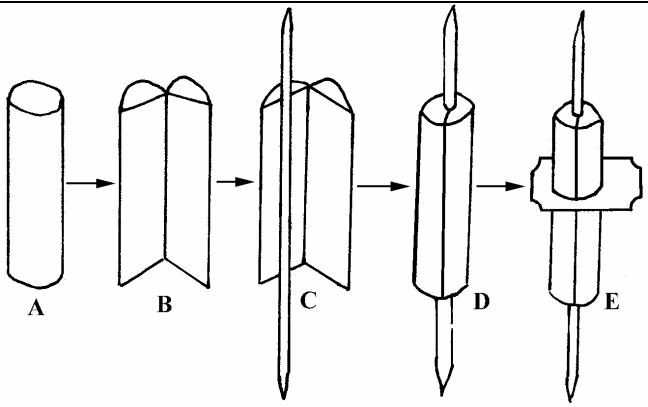


Figure 1. Preparation of cross-section of sample No. 9
A – Black elderberry pith
B – Cut black elderberry pith
C – Sample placed between cut black elderberry pith
D – Ready for cutting
E – Preparation of cross-section

Q 2 A. When the preparation is ready, using the low power x10 objective, find the best cross-section. Raise the card showing "2A", and the assistant of the laboratory task will assess the quality of the cross-section and preparation, and write the assessment points in the answer sheet!

(2 points)

Q 2 B. Using the microscope (objective x10 and x40), study the preparations. Compare the cross-section seen under the microscope with that in Figure 2. and with its fragments [A (Figure 3), B (Figure 4), C (Figure 5), D (Figure 6)].

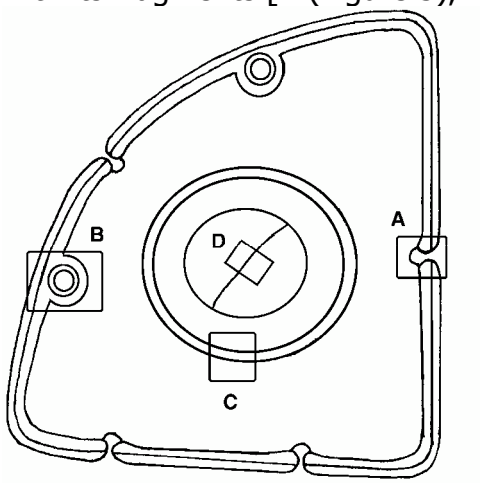
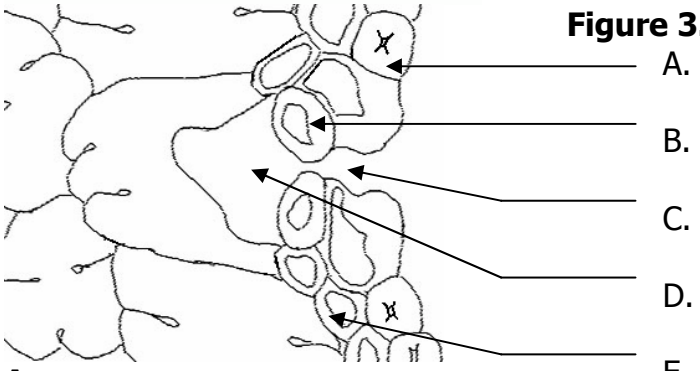


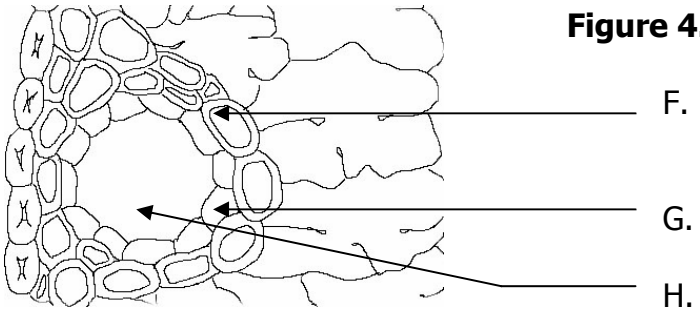
Figure 2. Cross-section of sample No. 9.

Figure 3. Sample No. 9 cross-section fragment A.



A.

Figure 4. Sample No. 9 cross-section fragment B



B.

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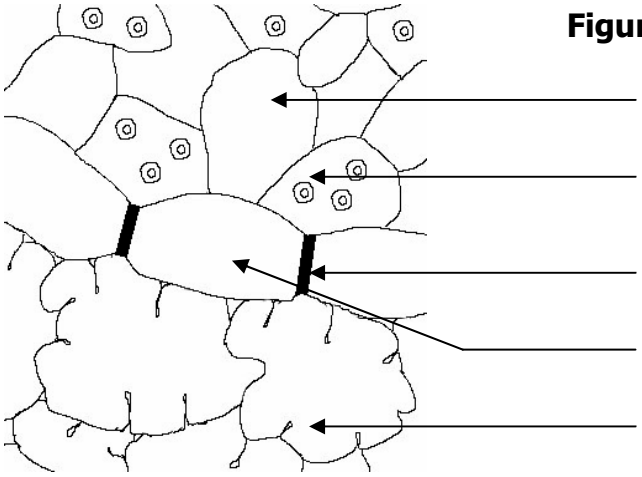


Figure 5. Sample No. 9 cross-section fragment C

C.

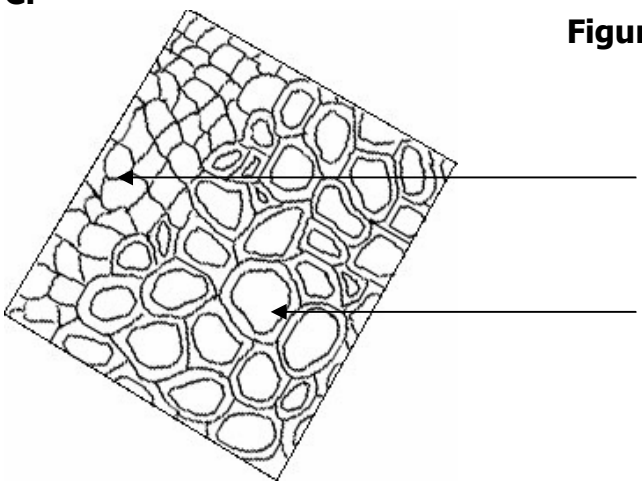


Figure 6. Sample No. 9 cross-section fragment D

D.

Code Table

No.	Part	No.	Part
1.	Pericycle	**13.**	Phloem
2.	Cystolith	**14.**	Transfusion tracheid
3.	Transfusion parenhima	**15.**	Palisade parenchyma
4.	Hypodermis	**16.**	Spongy parenhyma
5.	Casparian strip	**17.**	Guard cell
6.	Endodermis	**18.**	Sclerenchyma sheath
7.	Back cavity	**19.**	Raphide
8.	Trichome	**20.**	Angular collenchyma
9.	Epidermis	**21.**	Front cavity
10.	Druse	**22.**	Lobed parenchyma
11.	Resin duct	**23.**	Xylem
12.	Epithelial cells	**24.**	Pith

In the answer sheet beside the letters A-O of parts seen on Figures 3-6, write the codes of the correct names of these parts!

(15 points)

Q 2 C. Choose the correct plant taxon observed, and enter an "x" beside the respective code in the answer sheet.

Code table:

A.	Bryophyta
B.	Equisetophyta
C.	Pinophyta
D.	Magnoliophyta

(1 point)

Q 2 D. Choose the correct ecological group of the plant observed, and enter an "x" beside the respective code in the answer sheet.
Code table:

A.	hydrophyte
B.	hygrophyte
C.	mesophyte
D.	xerophyte

(1 point)

Task 3. Plant Physiology

Materials and instruments:
You will use the instrument set that you received upon registering for the 13th IBO!
You will also use other instruments and materials: sample No.10 – onion fragment, microscope, $Ca(NO_3)_2$ solution, distilled water, microscope slides and coverslips, razor blade, filter paper, cloth material.

A characteristic stem modification (bulb) fragment from a representative of the Liliaceae is supplied. Separate from the bulb fragment one fleshy inner scale leaf, using the instrument set!

Q 3 A. Determine on which side of the fleshy inner scale leaf can be found the lower epidermis. Lift the card with sign **"3A",** the assistant of the laboratory task will arrive, and you will show him the lower epidermis. His assessment will be entered in the answer page! ***(1 point)***

Q 3 B. Make a preparation**:** using a razor blade, shave a thin (~5 x 5 mm) section of the lower epidermis and place it on the microscope slide. Add one drop of the $Ca(NO_3)_2$ solution, place a coverslip over the section, and begin observation of the process occurring immediately under the x10 objective. Raise the card "**3B",** and the assistant of the laboratory task will arrive. His assessment of the preparation quality will be entered in the answer page! ***(1 point)***

Q 3 C. What is the name of the process seen under the microscope? In the answer sheet, enter an "x" beside the code of the correct process.
Code table:

A.	Hemolysis
B.	Dissociation
C.	Association
D.	Plasmolysis
E.	Deplasmolysis
F.	Hemophosphorylation

(1 point)

Q 3 D. Which of the below concentrations of $Ca(NO_3)_2$ solution could have caused the process observed? Enter an "x" beside the correct codes of the possible concentrations in the answer sheet.
Code table:

A.	1 M
B.	0.5 M
C.	0.1 M
D.	0.05 M
E.	0.01 M

(2 points)

4.1.3. Laboratory III – Molecular Biology

Length of the practical test - 60 minutes; 40 points

Task: Electrophoretic separation of plasmid pX DNA fragments in an agarose gel and construction of a restriction map of the pX plasmid.

The lab assistants will give **5 points** for strict following the lab safety regulations and accurate sample loading:

A - wearing the lab gloves during laboratory experiment – ***1 point***;
B - addressing the assistant before usage of the power supply and correct usage of UV transilluminator – ***1 point***;
C - proper use of pipette – ***1 point,***
D - loading the whole amount of the sample in the well - ***1 point***;
E - not damaging the gel – ***1 point***.

Note: One power supply is used by 3 - 4 – students, one UV transilluminator is used by 2 students!

Please wear the gloves during laboratory experiment !



Managing of power supplies is the priority of laboratory assistants !

Technical explanation

Theory

Electrophoresis is a widely used analytical method for separation of molecules by their charge, molecular weight and size. Frequently electrophoretical separation is performed in gel media where molecules with similar charges are separated according to their molecular weight and size. The substance, which forms the gel, has to be dissolved in the buffer solution.

Mapping of plasmid DNA

Plasmids are circular extrachromosomal double-stranded DNA molecules, which are found in many bacterial species. Restriction enzymes are nucleases, which cleave DNA at the sites where specific 4 – 6 nucleotide (base) pair (bp) sequences are found; e.g. enzyme called *Hae*III cuts the double stranded DNA at sequence (site) GGCC, but enzyme called *Eco*RI cuts the double stranded DNA at sequence (site) GAATTC.

Plasmid DNA mapping is placing of the restriction enzyme cleavage sites relative to each other on the circular scheme of the plasmid molecule. For this purpose we have to determine the length of DNA fragments produced by cleavage of the plasmid with different restriction enzymes. Plasmid molecules can be cut by one or by multiple restriction enzymes simultaneously. DNA fragments produced at cutting migrate as compact bands, which can be visualised in the gel by staining with specific dyes. The distance, which DNA fragment migrates in the gel during electrophoresis (cm from the start point of the migration till the front edge of the fragment band), is inversely correlated to the logarithm of the length of the fragment as measured in bp. One of the most common gel substances for electrophoresis is agarose. Pores of the agarose gel are large enough for separation of molecules with molecular mass over 100 000 Da.

Equipment

Agarose gel electrophoresis tank

(4, Fig.1). contains two electrodes - cathode (5) and anode (6), respectively. Before electrophoresis the gel is overlaid with buffer solution (7). Samples, which contain the mixture of molecules to be analysed, are loaded in the wells (1), which are formed by special comb during the preparation of the gel (2) on the gel support (3). Before connecting to the power supply the electrophoresis tank is closed with a cover (8).

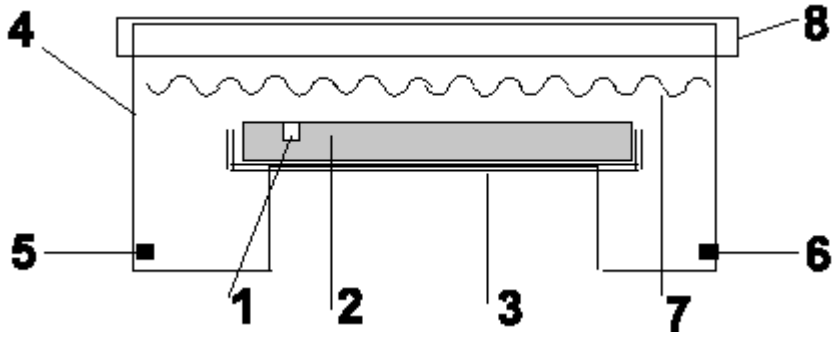


Fig 1. Electrophoresis tank with a gel.

Adjustable volume pipettes are used for handling of liquids (Fig.2).

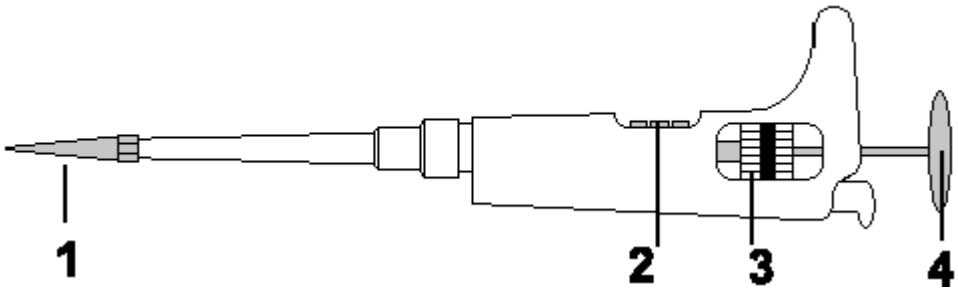


Fig 2. Adjustable volume pipette.

Use of the pipette:

1. By turning of adjustment ring (3) and controlling the volume monitor (2) set the appropriate volume! In this experiment you need to handle two volumes – 5 µl and 10 µl. Correct setting of these volumes on the monitor is shown in Fig. 3.

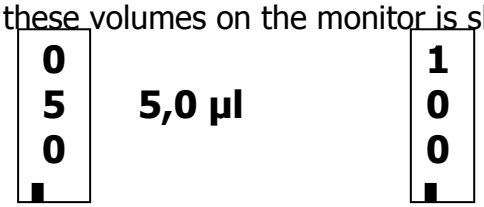


Fig. 3. Correct setting of 5 µl and 10 µl volumes on the monitor of the pipette.

2. Place the yellow tip (1, Fig.2) on the shaft of the pipette.

Do not handle liquid without a tip!

3. Press the button (4,Fig.2) smoothly to the first stop and put the tip in liquid (sample), (Fig.4,A).
4. Slowly release the button to aspirate the sample (Fig. 4 B).
5. Take the tip with the liquid to the target (other drop of liquid or well in the gel) and press the button until collected liquid is completely out of the tip (Fig. 4 C).

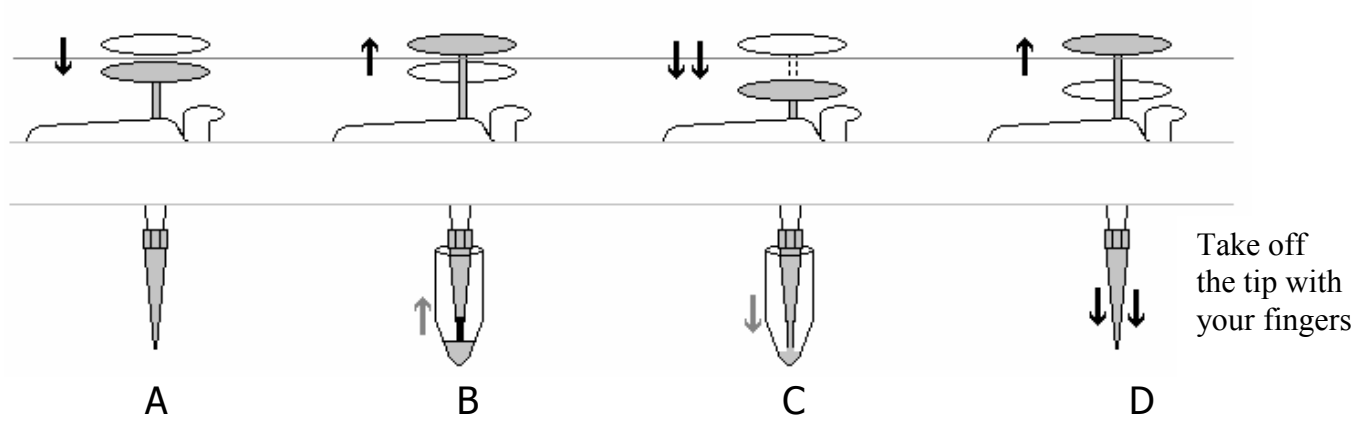


Fig. 4. Steps of liquid handling.

6. Take out pipette from the liquid, release the button (Fig. 4 D) and displace the used tip in the trash, labelled as:



For each solution or sample use a fresh tip!

You can make some trial pipetting attempts with one tip and buffer solution in the tank before starting to handle DNA samples.

Reagents and materials

1. Ready for use 0,8% agarose gel, prepared in 0,5x TAE buffer.
2. Electrophoresis unit, filled with 0,5x TAE buffer (20 mM Tris-acetate, 0,5 mM EDTA, pH=8,0).
3. 2x **GLB** - gel loading buffer, containing 0,05% bromophenol blue in 10% glycerol.
4. **St** - DNA size standard, premixed with loading buffer (more detailed explanation below)
5. **B+C** and **B+D** - 5 µl of plasmid pX DNA each, cleaved with restriction enzymes B + C ; B + D, respectively (detailed explanation see in **"Q2"** below).

To DNA samples fluorescent DNA dye Vistra Green in dilution of 1:10 000 is already added.
For all cleavages DNA of plasmid pX is used. The length of plasmid pX is 4 360 bp.

Experiment (first phase)

Sample loading

1. Load on the gel in each of the wells No.2 and No.5 (Fig. 5) 10 µl of DNA size standard **St.**

2. Add 5 µl of 2x **GLB** to each of cleaved plasmid DNA samples (**B+C** and **B+D**) and load the mix (10 µl) on the gel (**B+C** in the well No.3 and **B+D** in the well No.4, respectively).

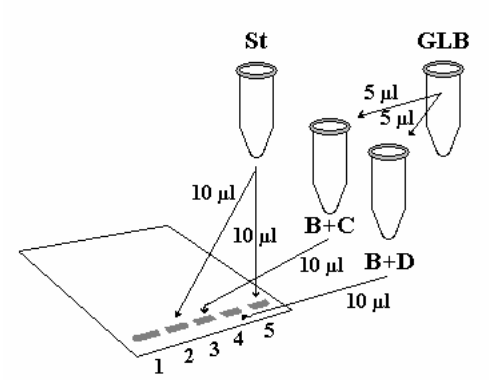


Fig. 5. Sample loading.

3. Close the cover of the electrophoresis unit. **Call the assistant by raising the hand!**



Do not manage power supply; this is the priority of laboratory assistants !

Let the samples run for 20 min. Mind the time, otherwise you lose DNA fragments! Use this time to prepare the answers to the questions below!

Questions (first set, to be answered while the gel runs)

Q1. It is known that in the electrophoresis buffer with pH 8,0 DNA molecules are migrating from cathode to anode.

Give the answers by marking the appropriate boxes **Q1** in the answer list.

- What is the charge of DNA molecules?

A. negative
B. neutral
C. positive
D. Impossible to determine

- Which of the mentioned components is the major determinant of the charge of DNA molecules?

E. purines
F. pyrimidines
G. deoxyriboses
H. phosphate groups
I. hydrogen bonds between the both DNA strands
J. No one of the mentioned

(2 points)

Q2. DNA fragment calculations

Given:

1. The picture of the gel showing electrophoretic separation of DNA size standards and DNA fragments produced by cutting of the plasmid pX with the restriction enzymes A, B, C, D (Fig. 6).
2. Size of the plasmid pX is 4 360 bp (base pairs) and each restriction enzyme (A, B, C, D), cuts the pX DNA at one site (one time) only.
3. The restriction site of enzyme A is taken as the starting point for restriction map of this plasmid.
4. In a combined cleavage with enzymes A and B DNA is cut in two fragments, shorter of which is 380 bp long (see Fig. 7).
5. Length of the DNA fragments in the bands of the DNA size standard (Lane 1, Fig. 6.):
   3 000; 2 000; 1 500; 1 200; 1 031; 900; 800; 700; 600; 500; 400; 300; 200; 100 (in bp)
   Band of 500 bp fragments has elevated width (is darker) in respect to neighbour bands.
   Bands of short DNA fragments (under 500 bp) may be weak or lost from the gel.

Estimate

Q2A. What is the size (bp) DNA fragments marked with the Roman numerals (I-VI) in the DNA size standard Lane 1, Fig.6.? Put the answers in the appropriate cells (I-VI) of the table Q2A in the answer list.

(3 points)

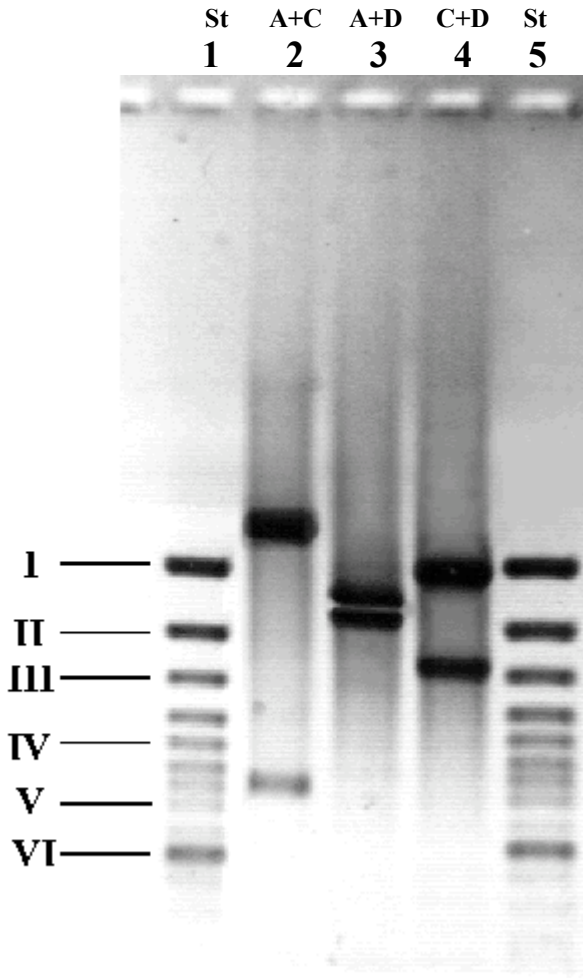


Fig. 6. Electrophoretic separation of plasmid pX cleavage fragments.
Lanes are numbered under the gel, the wells are seen in the upper part.
Lane 1 – DNA fragment length standard
Lane 2 cleavage of plasmid pX with enzymes A + C
Lane 3 cleavage of plasmid pX with enzymes A + D
Lane 4 cleavage of plasmid pX with enzymes C + D
Lane 5 – DNA fragment length standard

Q2B. Plot the distance (cm) migrated by the DNA fragment length standard bands marked with Roman numerals in Fig.6 versus the length of DNA fragments (bp) as determined in your answer Q2A in the co-ordinates Q2B in your answer list. Make the graph using the plotted points
On the X-axis - distance from the well to the front (distant) edge of the band (cm); on the Y axis – length of the DNA fragments (bp). ***(4 points)***

Q2C. Using the graph constructed in paragraph Q2B determine the size (bp) of DNA fragments presented in lanes 2, 3 and 4, Fig.6. Put the answers in the columns 2, 3 and 4 of the table Q2C in the answer list, corresponding the gel lanes 2, 3 and 4, respectively. (Allowed accuracy ±10% of exact value). ***(6 points)***

Q2D. In the sample A+C (Lane 2, Fig.6) after mixing with gel loading buffer DNA concentration was 150 ng/μl (nanograms per microliter), 10 μl were loaded on the gel.

How much DNA (in ng) was loaded on the gel? Put the answers in the column 1 of the table Q2D in the answer list.

How much DNA (in ng) is contained in each of the bands in the lane 2, Fig.6 (A+C) (assuming that all the loaded DNA is distributed between the two bands)? Put the answers in the column 2 (for the band of the largest DNA fragment) and column 3 (for the band of the smallest DNA fragment) of the table Question 2D in the answer list. (Allowed accuracy ±10%). ***(6 points)***

If you still have time until the gel is ready, you can start considering the possible location of cutting sites for the restriction enzymes C and D in the plasmid map (Fig. 7). Use the results from the answer to Q2C!

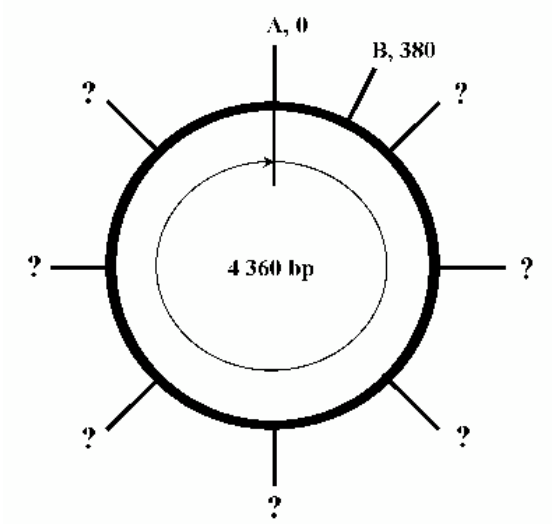


Fig. 7

Experiment (second phase)

20 minutes after the beginning of electrophoresis the assistant will disconnect your electrophoresis tank from the power supply. Do not hesitate to remind the time to the lab assistant!

Thereafter:

1. Place the gel together with the gel support in a tray and take them to the UV transilluminator, the number of which is indicated at your working place.
2. Pick up the protecting shield of the transilluminator.
3. Place the gel on the UV table.
4. Close the protecting shield and switch on UV light.

Do not switch on the UV transilluminator, while the protecting shield is open !



Do not lift the protecting shield while the UV light is on !



5. Observe the image of DNA bands and draw the pattern of bands in the frame given in the answer list **Q3**. DNA bands in your picture must be positioned relative to the DNA size standard precisely as in your gel!
(4 points)

6. Switch off the UV light and leave the gel together with gel support at the transilluminator.

7. Clean the hands with paper towel and continue preparing your answers.

Questions (second set, to be answered after the recording the fragment separation in the gel)

Q 4 A. Determine the approximate fragment length of cut DNA, comparing the position of the bands of samples with the bands of DNA size standard. Put the answers in the answer list; table Q4A, columns 3 and 4, corresponding the lanes 3 and 4, respectively. (Allowed accuracy ±20%).
(4 points)

Q 4 B. Considering the analysis of the gel depicted in Fig.6 and the data obtained from your own gel, determine the approximate positions of the cutting sites of the enzymes C and D at the plasmid map (Q4B, answer list) by writing the letters (C, D) in appropriate boxes. ***(6 points)***

4.1.4. Laboratory IV – Dendroecology

Growth of aspen (*Populus tremula*) invading a clear cut (previously spruce) by seed.
Length of the practical test – 60 minutes; 40 points

Materials and instruments

You will need the instrument set that you received upon registering for the 13th IBO!
Other materials:
A data sheet. The data sheet is identical to the answer sheet with tables, only with some of the columns (Table 1) and rows (Table 2) filled in. THIS DATA SHOULD BE COPIED ONTO THE ANSWER SHEETS. DO NOT WRITE ON THE DATA SHEETS.
10 labelled discs of aspen. DO NOT MAKE ANY MARKS ON THE DISCS
one measuring tape
one ruler
one magnifying glass

Introduction

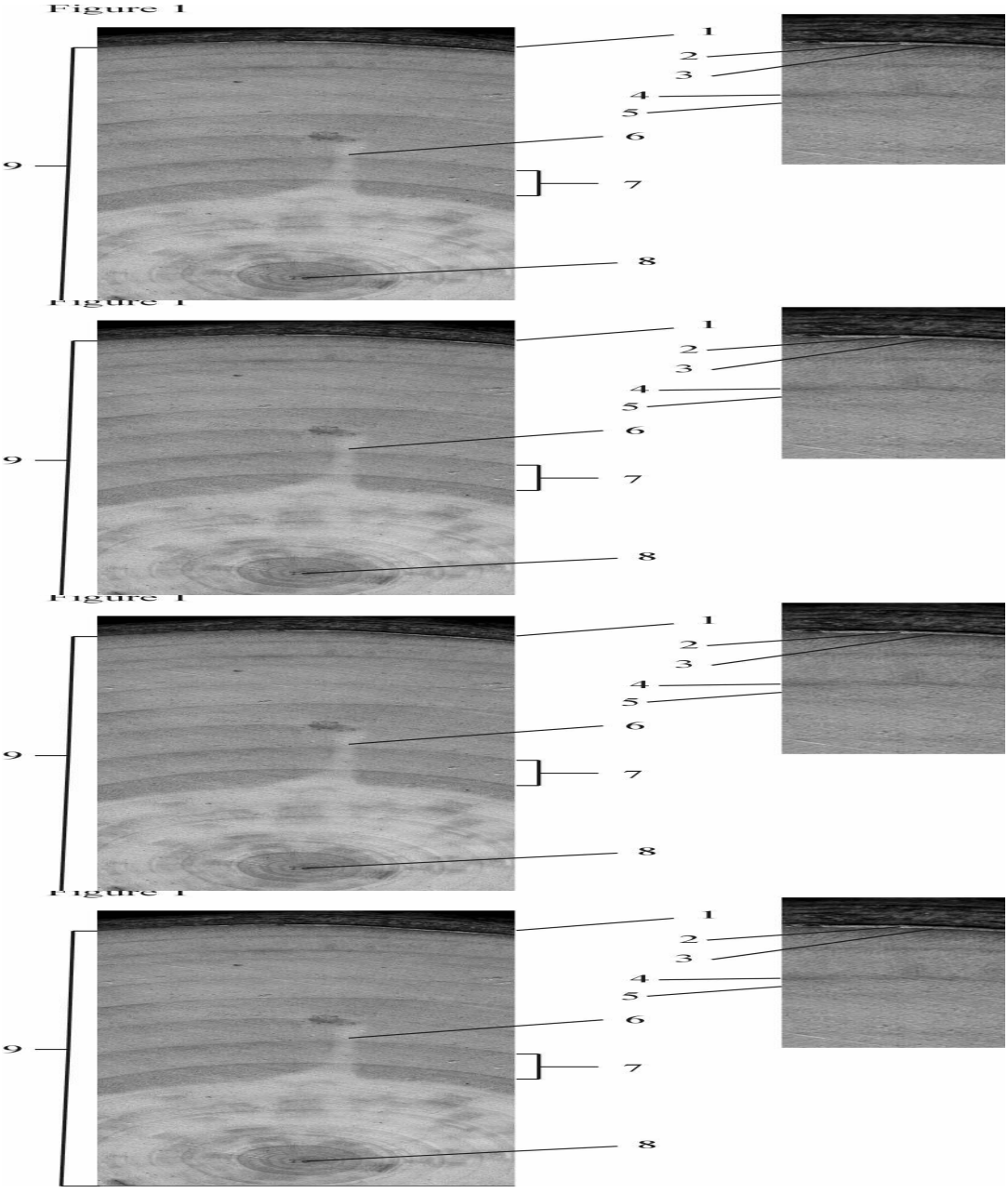
Aspen has invaded an open area created by a clear cut harvest of a spruce forest. Not all of the trees established in the same year, but over a 8-year period. Now, 18 years after the start of development of the new forest stand, the vertical and radial growth of aspen will be investigated by retrospective analysis. Tree rings can be used to measure the tree stem diameter at different tree ages.

Q1. In the following photograph (Figure 1) of a cross-section of an aspen stem, identify the following, by writing the respective number from the Figure in the answer sheet!

Code Table

A.	bark
B.	pith
C.	latewood
D.	earlywood
E.	meristem
F.	annual ring
G.	branch
H.	vascular cambium
I.	phloem cells
J.	xylem cells
K.	cork cells

(11 points)


Figure 1
1
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6
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Figure 1
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Figure 1
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Figure 1
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The growth of aspen stems in the area will be used to determine response to possible intraspecific competition. It is assumed that the effect of competition increases with time, because tree density increases and the trees increase in size.

Q2. For a tree species, not necessarily aspen, what are the **possible** effects of intraspecific competition for light resources:
1. increased mortality rate over time,
2. decreased yearly growth increment in height of stems
3. increased variability among stems of yearly growth increment in height
4. decreased yearly growth increment in diameter of stems
5. increased variability among stems in yearly growth increment in diameter
6. accelerated yearly growth increment in height of stems in response to shading by neighbours

Which of the following is the correct combination of correct answers? Enter your choice in the answer sheet!
A. 1, 2, 4
B. 1, 3, 5
C. 1, 2, 4, 6
D. all of the above
E. 1, 2, 3, 4, 5

(1 point)

The purpose of this practical work is to determine whether radial and/or vertical growth of aspen is related to time of invasion of aspen individuals. In other words, since the time of invasion can be determined by estimating the age of stems, does the growth depend on the age of the individual?

Methods and Results
Within a forest plot (20mX20m), all trees growing were cut at tree base, labelled and stem height was measured. Each student is supplied with 10 segments cut at stem base, which were randomly sampled in the plots. Each stem is labelled, and the height of these stems in metres is provided beside the appropriate tree ring code in **Table 1** of the data sheets. **Copy the codes and the heights onto the answer sheets.**

T1. **Fill in the** Table 1**!**

- Count the number of tree rings of each segment, and enter this data in Table 1 of the answer sheets! ***(2 points)***

NOTE: Since determination of the first few tree rings is difficult due to rot, the first five tree rings developed have been determined under a dissecting microscope, and the position marking the end of five years is marked on each stem. Therefore, you need only to count the rings developed after this mark, and then add 5 years.

- As none of the cut segments are perfectly round (the radial growth differs depending on compass direction), the mean diameter will be estimated by calculation from the perimeter. Using the cloth tape provided, measure the perimeter of each stem, and enter this data in **Table 1** of the answer sheet! ***(2 points)***
- Using the appropriate formula, use the perimeter measurements to calculate the mean diameter, and enter this data in **Table 1** of the answer sheet! ***(1 point)***

- Using the ruler provided, measure the total width of the first five tree rings (from the centre to the mark on the segment) and enter this data in **Table 1** of the answer sheet! ***(1 point)***

- Using the ruler provided, measure the last five tree rings (at the location of the maximum diameter (marked by the line), and enter this data in **Table 1** of the answer sheet! ***(1 point)***

G1. Using the graph paper supplied in the answer sheet, produce scatter plot for **Graph 1.** - Stem age versus height ***(2 points)***

G2. Using the graph paper supplied in the answer sheet, produce scatter plot for **Graph 2.** - Stem tree age versus diameter ***(2 points)***

G3. Using the graph paper supplied in the answer sheet, produce scatter plot for **Graph 3.** - Stem age versus width of first produced five tree rings ***(2 points)***

G4. Using the graph paper supplied in the answer sheet, produce scatter plot for **Graph 4.** - Stem age versus width of the last five produced tree rings. ***(2 points)***
Remember to label the axes with the appropriate scales.

G5. As the graph of stem age versus height appears to indicate a linear relationship, calculate the linear regression equation (best-fit line through the points) using the Tables provided (**Table 1** and **Table 2**) and draw the calculated best-fit line on the appropriate graph ***(1 point)***

NOTE: **In the data sheet supplied, you have been given the correct values of the sum of squared ages (ΣXi^2) and the value of the sum of age times**

height (ΣXi*Yi). Therefore, you do not need to calculate these. ENTER THESE FROM THE DATA SHEET ONTO THE ANSWER SHEET.

T2. Completely fill in **Table 2** on the answer sheets ***(8 points)***

Check that **Table 1** is completely filled in.

Discussion

Q3. The **Graphs** that you have produced suggest that intraspecific competition has resulted in:

1. reduced height of shaded individuals
2. reduced diameter of shaded individuals
3. reduced stem structural support of shaded individuals
4. increased variability in height of individuals of the same age
5. Increased variability in diameter of individuals of the same age

Which of the following is the correct combination of correct answers?

A. 1, 2, 3, 4, and 5
B. 1, 2 and 3
C. 2, 3 and 5
D. 2 and 4
E. 4 and 5

(1 point)

Q4. Which of the following comments are suggested by the **Graphs** that you have produced?

1. The reduced growth of individuals invading the clear cut later suggests that aspen has a stress-tolerant growth strategy.
2. The **Graphs** suggest a certain competitive ability, because late-coming stems are able to maintain the same growth rate in height as the stems which arrived earlier, indicated by the linear relationship between age and height.
3. In combination, the **Graphs** suggest that there will be potentially increased mortality of the individuals invading the stand later.
4. The **Graph 4** suggests that the growth strategy of aspen appears to be a ruderal strategy: rapid growth early in succession, taking advantage of available resources

Which of the following is the correct combination of correct answers?

A. 1 and 3
B. 2 and 4
C. 2, 3 and 4
D. 4

(1 point)

Q5. Which of the following comments are suggested by **Graphs 3 and 4**?

1. The **Graphs** show that the effect of competition increases with time after clearcut.
2. Only the trees arriving on the site during the first few years (but not necessarily all of these individuals) have been able to support a high radial growth rate during the last few years prior to cutting.
3. Differences in the amount of shading in the occupied patches may be a reason for the high variability in **Graph 3**.
4. Graphs 3 and 4 probably reflect linear relationships that are hidden by high variability.

Which of the following is the correct combination of correct answers?

A. 1, 2 and 3
B. 4
C. 1, 2, 3, and 4
D. 1 and 3
E. 2 and 4

(1 point)

Q6. Which of the following problems clearly need to be considered, as they can affect the results shown in the **Graphs**?

1. The annual growth increment in tree ring width is related to the current stem diameter. For this reason, calculation of the relative growth rate (RGR) would have less bias.
2. Differences in the growth of the different stems should also be assessed in relation to differences in meteorological conditions during the years after the clear cut.
3. Biotic factors such as herbivory (insects, deer, moose which do occur in the area) and disease may have caused death or damage to a particular age class of stems, biasing the results.
4. The sample size is too low for conclusive results.

Which of the following is the correct combination of correct answers?
A. None of the comments are true
B. All of the comments are true
C. Only 1 and 4 are true
D. Only 2, 3, and 4 are true
E. Only 2 and 3 are true

(1 point)