SAPS SCIENCE & PLANTS FOR SCHOOLS

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Plant biotechnology . . .
at The Scottish Crop Research Institute

The Scottish Crop Research Institute (SCRI) is located in Invergowrie, near Dundee. SCRI is a major international centre for basic, strategic and applied research into crop-based bioscience and related environmental sciences. Staff include graduate scientists, visiting workers and research students and links are maintained with 300 Institutions worldwide.

Research at SCRI is organised into broad themes: Mechanisms and Processes, Genes to Products and Management of Genes and Organisms in the Environment. Research focuses on three main crops (potato, barley and soft fruit) and a number of pathogens and pests, with additional work on a wider range of temperate crops.

Aerial view of the Scottish Crop Research Institute (SCRI), an important centre for research in plant biotechnology and improvement of crop plants *© SCRI (2003)*

Plant biotechnology

The term 'biotechnology' was first used in 1919 and covers many aspects of modern biology, chemistry and environmental studies. Plant biotechnology has the potential to generate valuable products such as nutritionally enhanced foods, safe, cheap and effective plant-based pharmaceuticals or plants with environmental advantages. Plants are highly efficient natural refineries and large quantities of valuable products can be produced in small areas safely and relatively cheaply.

It may be surprising to learn that 90% of the world's population relies on only 15 crop species for their food. These species include the cereals - rice, barley, wheat, rye, maize, sorghum and millet - together with coconut, potato, cassava, soya bean, groundnut, sweet potato, faba bean and banana. For much of the past two centuries, science and good farming have enabled food production to keep pace with population growth. Major advances in plant biotechnology during the last 25 years have widened the scope and precision of crop plant improvement but today, increases in crop yields (of about 1% per year) are lagging behind the 1.8% increase per year in the human population. This 'production deficit' is widening due to factors such as climate change, erosion, pests and diseases. Political pressure is another factor that can prevent significant expansion of agricultural production.

Modern plant biotechnology is largely based on two key technologies: the isolation, cloning and transfer of DNA and the ability to regenerate plants from single cells or pieces of tissue. Progress in molecular genetics, including gene discovery and sequencing, has led to the use of plants for the 'manufacture' of a wide range of recombinant DNA products. By 2002, over 50 million hectares (an area approximately the size of Spain!) of genetically modified (GM) plants were being grown worldwide. These crops were mainly soya, cotton, maize and oilseed rape, and most were grown in the USA, Canada, China and Argentina. None were grown in Europe, other than those for experimental trials.

Initially, genes inserted into GM crops conferred traits such as herbicide or pesticide resistance. Current worldwide research is aiming to produce plants with other benefits, including

- improved nutritional qualities (e.g. "Golden Rice")
- high value "pharming" where plants are used as factories to produce antibodies, vaccines, diagnostics, vitamins or entirely new synthetic drugs.

Science and Plants for Schools, Homerton College, Cambridge CB2 2PH Tel: 01223 507168 www.saps.org.uk
SAPS Biotechnology Scotland Project, c/o SSERC, St Mary's Building, 23 Holyrood Road, Edinburgh EH8 8AE Tel: 0131 558 8212

While GM techniques are potentially important, plant biotechnology includes useful technologies that do not involve gene transfer from other organisms. These include the following:

- **Micropropagation** - a scaled-down version of growing new plants from cuttings. In the laboratory over 1 million exact copies of the plant can be generated in one year! SCRI uses micropropagation to speed up breeding programmes. In the potato industry in Scotland, 65% of seed potatoes now originate from test tubes.
- **Cell suspension cultures** - these are a plant "soup" of selected cells, used to generate high value products such as anti-cancer drugs, colours and flavours. In Taxol research for example, the use of suspension cultures derived from living yew trees allows mass production of the drug. Without this technology, six one-hundred-year-old plants would be destroyed to treat one patient. At SCRI cell suspensions of such species as potato, tobacco and barley are widely used in research programmes.
- **Protoplasts** - each leaf of a plant contains over a million cells. Leaves can be treated with enzymes to strip away the cell walls and produce protoplasts, which are effectively 'naked' plant cells. At SCRI, protoplasts are used routinely in studies into cellular development. Protoplasts can also be fused with cells of another species producing somatic hybrids - an important scientific tool with potential as a non-GM means of crop improvement.

Research programmes at SCRI are directed towards understanding the fundamental mechanisms underlying plant growth and differentiation, and pest and disease resistance.

GM and geneflow

Gene-flow is not a new concept as crops and arable plants (weeds) have competed and exchanged genes since the beginnings of agriculture. The arable plants are an important part of the biological structure of arable fields. They support more invertebrates (many benign or beneficial) than crop species and provide diversity of material for food webs in the soil. It is essential to reach a balance that allows continued food production to meet our needs whilst maintaining a resilient, sustainable habitat.

At SCRI, studies with oilseed rape (*Brassica napus*) have looked at evolution in fragmented populations. Measuring and predicting crop purity has become necessary following applications by seed companies to grow GM crops in Europe. SCRI was part of the independent consortium involved in farm-scale evaluations of herbicide-resistant GM crops for the UK government, looking at the effects of these crops on arable food webs, feral populations, competition and gene exchange with feral, wild or nearby crop species. For more information on the farm-scale evaluations, see *Further reading*.

SCRI and its links with education

The speed at which new advances are occurring in all areas of research means it can be difficult to keep

Bee on oilseed rape. Studies with oilseed rape* (Brassica napus) *have made useful contributions to the GM debate - SCRI has been involved in farm-scale evaluations of herbicide resistant GM crops
© SCRI (2003)

science teaching up-to-date. SCRI is committed to helping teachers by providing a service that includes access to information, meeting with SCRI scientists and demonstrations of equipment or techniques to staff and pupils. SCRI has a full-time Education Officer, who works with children, teachers, other educators and the general public to increase interest in and knowledge of today's science. SCRI is also a LEAF (Linking Environment and Farming) Innovation Centre, pioneering and publicising new approaches in sustainable land management.

For more information about SCRI, you can contact Sharon Neilson, (SCRI Education Officer, email: S.Neilson@scri.sari.ac.uk); Sarah Stephens, (Science Communications, email: S.Stephens@scri.sari.ac.uk); Dr Steve Millam (Plant Biotechnology and its role in human health and nutrition, email: S.Millam@scri.sari.ac.uk)

A sixteen year old "Nuffield Bursary" school student, carrying out a 6-week laboratory based project, during the summer of 2003, with assistance from SCRI scientists ***© SCRI (2003)***

Further reading:

SCRI: www.scri.sari.ac.uk
DEFRA (GM): www.defra.gov.uk/environment/gm/index.htm
Golden Rice: www.biotech-info.net/golden.html
Plant Molecular Farming Discussion (Canada):
http://www.inspection.gc.ca/english/plaveg/bio/mf/molecule.shtml
Farm-scale Evaluations:
www.defra.gov.uk/environment/gm/fse/
LEAF: www.leafuk.org
The Nuffield Foundation: http://www.nuffield.org/award

Sharon Neilson (Education Officer)
Scottish Crop Research Institute, Invergowrie

HEAD OFFICE
Homerton College
Cambridge CB2 2PH
Tel: 01223 507168
Fax: 01223 215004

STUDENT SHEET 25

The wonderful world of wee things . . .
a microworld in a hanging drop

Do all microorganisms look the same? Can they move and change shape? What do they feed on? How small **are** they . . . and are they actually **alive**?

One way to find answers to these questions is to use a microscope to look at some microorganisms. In this practical activity, you will make a 'hanging drop' preparation then use a microscope to look at what is inside the hanging drop.

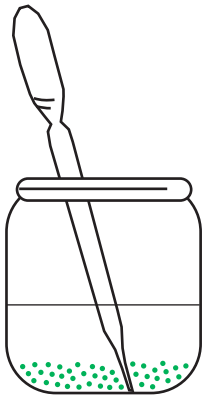
You need to collect two clean glass slides, a transparent film can lid, a piece of blu-tak, a pipette or dropper, a bottle of mixed algae and a microscope. Then follow the steps shown in the diagrams in the boxes.

1

MS = Microscope slide
L = Film can lid (transparent)

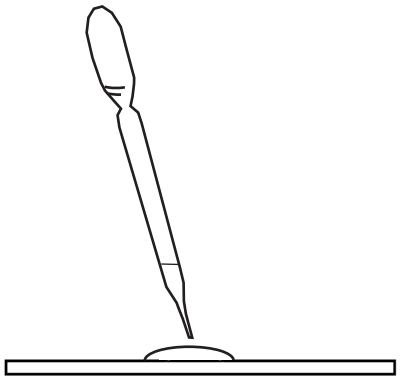
- Attach the film can lid to one slide
- Place 2 small pieces of Blu-tak on rim of the film can lid

2



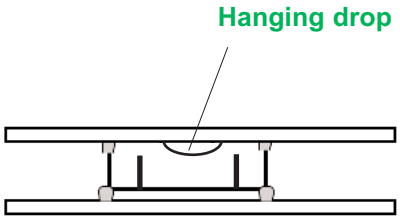
- Draw some algae from bottle into the pipette. Make sure you pick up some green stuff

3



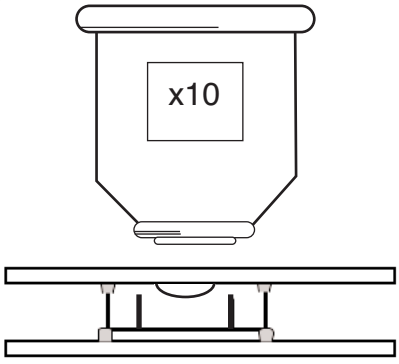
- Release a single drop of algae into the middle of the second slide

4



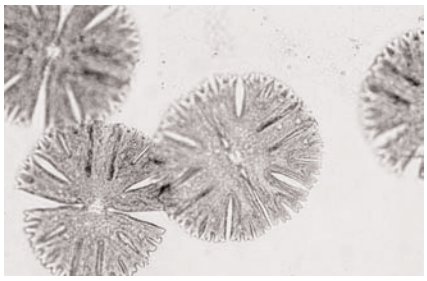
- Turn the slide over quickly and let the drop hang into the film can lid

5



- Place the slide on to the stage of the microscope
- Focus the microscope and observe the cells

6



Micrasterias* sp.** ***© Sciento (2003)

(See the SAPS website for more colourful images that you find in different cultures - coming soon!)

Notes for Teachers (*Student Sheet 25*)

The hanging drop technique is a well-established method for examining living, unstained, very small organisms. The traditional procedure employs a glass slide with a circular concavity in the centre into which a drop of fluid, containing the 'microorganisms', hangs from a coverslip. Cavity slides are expensive and coverslips are fragile so some pupils can find them fiddly to work with. Here we offer an alternative technique that is easy to use in the classroom. The simple substitute of a transparent film can lid, blu-tak and two microscope slides gives a cheap and practical option which allows pupils to look at living cultures easily and effectively. Algae and protozoa are of sufficient size for pupils to view them successfully using a standard school microscope. Examination of such 'hanging drops' can lead to useful discussions of size, variety, characteristics and importance of microorganisms and to consideration of differences between 'plant' and 'animal' cells.

The aim of this activity is to allow pupils to experience the magnificence of the microworld that can exist in a hanging drop and to observe some of the microorganisms that populate fresh water. When viewing a correctly focussed hanging drop preparation of the recommended mixed algae, using lenses to give x 100, x 200 or x 400 magnification, pupils are able to watch a wide variety of (mostly unicellular) algae of different sizes. Some are motile and swim across the field of view with amazing rapidity. Others, such as the desmids, which possess three perfect planes of symmetry, exhibit interesting and remarkable shapes, as do diatoms whose individual cells demonstrate astonishing intricate architecture.

You can use this method to study microorganisms in a 'bought-in' preparation, in pond water or in 'home-grown' cultures. We recommend mixed algal and mixed protozoal cultures obtained from Sciento. (We have found these Sciento cultures to be of consistently high quality both in variety and numbers of organisms.) In the case of the algae, make sure that the drop contains some obvious green material to allow observation of a variety of different cells and non-motile organisms (although a drop of 'clear' liquid may contain a limited variety of motile organisms such as *Chlorella*). When removing a sample of protozoa from the jar, it is important to select some of the solid material as the protozoa are likely to be feeding there.

All algae can carry out photosynthesis. This activity can, therefore, lead into discussions of the importance of the algae in carbon fixation in rivers, lakes (or lochs), seas and oceans and also of plant plankton as the producers at the start of food webs in water environments. Diatoms are thought to be an important constituent in the formation of oil deposits.

How can you find out the actual size of the organisms?

You could ask pupils what they think is the **actual** size of the algae. Then see what ideas they have as to how to measure the cells. They may suggest comparing the microorganisms to the width of a hair and examining them side by side under the microscope. To do this, remove the apparatus from the stage of the microscope and quickly invert it. Then remove the slide with the film can lid. Place a hair across the drop and carefully lower a coverslip over the drop and the hair. Pupils can then compare the width of the hair with that of the microorganisms. They can estimate the width of the hair by laying it on a ruler and judging size on the millimetre scale. A reasonable approximation can be made using a clear ruler under the microscope. Another idea is to photocopy graph paper (with mm squares) onto thick acetate and cut it to make graduated 'slides'. Then just hang the drop from these acetate graduated slides.

Suggestions for further activities

Examination of algae provides only a partial picture of the protoctista. This can be made more complete by preparing and examining hanging drops of cultures of protozoa. Pupils will see that most of the protozoa are not green, that they move by a variety of methods and that they can change shape. Pupils should be able to relate the ability to change shape to the presence or absence of a cell wall and then to their knowledge of animal and plant cell structure. Depending upon the microscope, they may be able to watch the organisms feeding and even see that some have cilia, which create 'currents' to 'draw in' foodstuffs. Pupils can measure the size of protozoa by one of the methods suggested above. These activities are suitable for use with a videocam.

Contact for Sciento: 61 Bury Old Road, Whitefield, Manchester M45 6TP (Tel: 0161 773 6338)

Discussion from pupil questions (see Pupil guide - Questions and More questions)

1 *Pupils may mention different sizes / shapes / symmetry / green colour*
2 *Hopefully they will answer "Yes" (because some can move) . . / possibly "No" (because they do not move)*
3 *Green colour*
4 *Hopefully - colour and ability to change shape*
5 *Hopefully they may refer to ability to change shape linked to presence / absence of cell wall; colour linked to ability to carry out photosynthesis)*

This practical work can be used to support the curriculum at Key Stage 2: 'Living things in their environment' and the level E attainment target 'Variety and characteristics of microorganisms' in the Scottish 5 - 14 Guidelines.

Pupil guide (*Student Sheet 25*)

At first it may be difficult for you to find the microorganisms under the microscope. You may need to take a little time to practise focussing the microscope until the image becomes sharp. Gently turn the focussing knob backwards and forwards so that you can focus up and down through the hanging drop. When you do this, you may see the microorganisms in three dimensions. Look carefully at the microorganisms and draw some of them, then answer the following questions.

Questions

1. How would you decribe in words the size and shape of the microorganisms that you see down the microscope?
2. Do you think the microorganisms are alive? Say why you think that what you do.
3. What feature of the microorganisms suggests that they could carry out photosynthesis?

When you have looked at the algae and answered the questions above, make a hanging drop of protozoa and look at them under the microscope. Draw some of them and then answer the next group of questions.

More questions

4. Look for two differences between algae and protozoa and write down your answers.
5. Can you link the differences in appearance or other features you can see in the algae and protozoa with what you know about the structure or function of plant and animal cells?
6. Try to think of something you could do to measure the actual size of the algae and protozoa. (Clue - can you think of something very thin that you could look at with a microscope for comparison?)

We hope you have enjoyed looking at some tiny organisms in what we have called a 'microworld in a hanging drop'.

Kath Crawford
SAPS Biotechnology Scotland Project & SSERC

News from SAPS

Personnel changes

Over the past few months we have had a number of changes in personnel within SAPS. Kirsty Menzies, who has been with the SAPS Biotechnology Scotland Project since October1995, left us at the end of September 2003 to embark on an MSc programme in *Information and Library Science*. Kirsty has been a pivotal member of the SAPS programme and she will be sorely missed. We are very fortunate to have been able to secure the services of Anne Adams at SSERC, together with additional support from Jane Inglis at Dollar Academy, and so we hope that "normal service" will continue to be the order of the day! Kath Crawford who has been full-time with SAPS since September 2000 is now spending half of her time on the 5-14 Improving Science Education programme within SSERC. We are fortunate that Pam Ferguson and Lucy Payne, both at Dollar Academy, have agreed to take on the delivery of some of our workshops.

Many of you will now be familiar with Debbie Eldridge's contribution to the SAPS programme (see http://www-saps.plantsci.cam.ac.uk/worksheets/ssheets/ssheet23.htm) from her work on photosynthesis. We are delighted to say that Debbie is now working with us for one day per week for the foreseeable future and we hope that she will be able to run some workshops together with spending time on curriculum development.

A new base for SAPS in Scotland

The SAPS office, previously located in the Institute for Cell and Molecular Biology at the University of Edinburgh, has recently moved to new accommodation at the Scottish Schools Equipment Research Centre (SSERC). Members of the team can be contacted at:
SAPS Biotechnology Scotland Project, SSERC, St Mary's Building, 23 Holyrood Road, Edinburgh EH8 8AE
(tel: 0131 558 8212; fax 0131 558 8191). There is a new general e-mail address for SAPS in Scotland:
saps@sserc.org.uk

Schoolteacher Fellowship

Robinson College at the University of Cambridge, in association with SAPS, is able to offer an annual fellowship in plant science for teachers in UK secondary schools and colleges. For the academic year 2003/2004 funds have been secured to allow for a teacher to spend one term at Cambridge working on a project related to the work of SAPS. The full cost of the Fellow's salary for the term is provided together with a grant for consumables and equipment. Accommodation within Robinson College is provided for the duration of the Fellowship. Initial expressions of interest to Paul Beaumont at the Cambridge office please.

Questions about Quadrats!

Interdependence is one of the five key science ideas at KS3. Whilst this can be taught theoretically, some practical fieldwork greatly enhances pupils' understanding. Almost certainly the fieldwork would entail looking at plant abundance and distribution and, of course, some work with quadrats!

A quadrat is a simple device for marking out a small area. For young children at primary school the quadrat is often a convenient way of focusing a pupil's attention on a particular small area. At secondary level, pupils should understand how quadrats can be used to sample a larger area. By recording information from a number of quadrats placed within a larger study area, they can obtain a representative sample of the whole area, which may be too big to describe in full.

This article describes how quadrats can be used to help pupils at lower secondary level estimate the relative abundance of plant species. All the information given here refers to frame quadrats. (Point quadrats can be tedious and difficult for pupils to use and are probably best avoided at this level.)

When using quadrats, here are five questions we need to answer.

1. How is the study area chosen?
2. What size and shape should the quadrat be?
3. What should be recorded within the quadrat?
4. What strategy should be used for placing the quadrats?
5. How many quadrats need to be placed?

1. How is the study area chosen?

Choose an area that is large enough to be representative of the vegetation being investigated. You also need to consider the time available for the study and the number of pupils involved. The area must not be so big that it cannot be sampled adequately or so small that the habitat is damaged by trampling feet .

Generally a plot size of about 20 x 20 m is suitable for a class of 25 to 30 pupils. Correct identification is crucial to all ecological work. If identifications are incorrect, it becomes impossible to explain results. It is best, therefore, within the study area, to limit the selection to a few plants that are easy to recognise. You can easily make identification sheets for students by scanning or photocopying actual specimens.

The fold out chart series produced by the Field Studies Council offers useful help in the identification of plants in a wide variety of habitats including woodland, grassland and heathland.

2. What size and shape should the quadrat be?

In theory any shape of frame can be used but for many measurements you need to know the area of the quadrat so a square quadrat is the most popular.

The size of the quadrat is usually related to the size of the plants being studied. Here are some useful guidelines, given in the Open University Project guide.

- 10 cm x 10 cm quadrats - for very small plants, such as algae or bryophytes on tree trunks or walls.
- 25 cm x 25 cm quadrats - for short grassland and other low-growing vegetation
- 50 cm x 50 cm quadrats - for long grass or heathland.

Larger quadrats are difficult to handle and for plants such as trees and shrubs it is probably best to mark out plots on the ground with tape measures.

Making the quadrats

The easiest way to make a quadrat, approximately 25 cm x 25 cm, is to bend a metal coat hanger into a square. Cut off the hook and for safety cover the cut end with insulating tape. To make a larger quadrat, purchase stock wire from an ironmonger or farm supplier. You can then bend this into quadrats of any size required. As for the coat hanger, cover the joined ends in insulating tape. Use a brightly covered tape so that quadrats left lying on the ground are more easily found! To make small quadrats, e.g. 10 cm x 10 cm (for using on a flat surface like a wall), draw the shape on an acetate sheet.

3. What should be recorded within the quadrat?

Abundance means the amount of something. Pupils are often asked to make an estimate by eye of the percentage amount of ground covered by each species within the quadrat. This can be time consuming and such subjective measures are very prone to inaccuracies, especially with younger pupils. It is better to carry out one of the quantitative measures described below.

When working with plants, the two measurements of abundance commonly used are:

- the number of individual plants
- the area covered by the overground parts of the selected species

We will look at each in turn together with an example to show how the measure is used.

The number of individual plants - The pupil counts the number of individual plants of the selected species in each quadrat. The result can be expressed as number of plants per square metre. This measure is known as **density**.

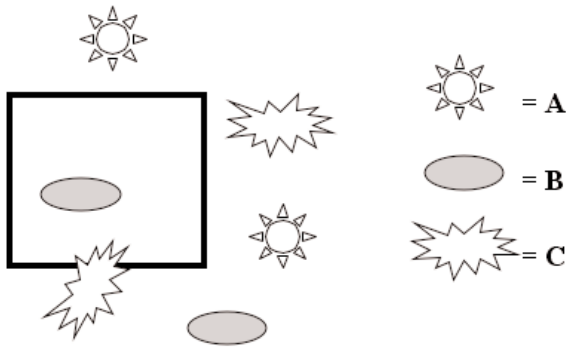
Example
The chosen study area measured 10 m x 10 m (100 m^2). Pupils placed 8 quadrats, each 50 cm x 50 cm (a total of 2 m^2).
A total of 24 daisy plants were found in the quadrats.
So there were 12 daisies per m^2.

This is an easy concept for pupils to understand but has disadvantages. Individual plants are often not easy to distinguish, e.g. grasses. Even plants that appear separate may be joined underground. No information is obtained about the size of the plants but this may be of great importance ecologically.

The amount of area covered by the overground parts of the selected species - The greater this area, the more likely a plant is to occur within a quadrat. This measure is known as **plant frequency** and, ecologically, is a more useful measure than density, as both the size and number of individual plants contribute to the area covered.

Plant frequency is also quicker to measure than density. It is not necessary to measure the *number* of plants of each species within a quadrat but only to record their presence or absence. A species counts as present if any part of the plant lies within the quadrat.

Results are usually expressed as the number of times a species occurred in the quadrats as a percentage of the total number of quadrats placed.



Species B and C are present in the quadrat but species A is not

Example
Daisies occurred in 15 of the 25 quadrats placed in the study area. Therefore, the percentage frequency of daisies is

$$\frac{15 \times 100}{25} = 60\%$$

The size of the quadrat obviously affects the result and so the same size of quadrat must be used in the areas being compared. If the two areas being compared have very different sized plants it is probably best to use the quadrat size best suited to the taller vegetation.

Local frequency is a useful measure when working along a transect line (see below). For each station along the line a frequency figure can be obtained by using a "gridded quadrat". The number of small squares that each species occurs in is expressed as a percentage of the total number of squares in the whole quadrat. It is common to use a 50 x 50 cm quadrat, divided into 25 smaller squares. You can easily make these quadrats by using plastic mesh (purchased from a garden centre) and cutting it to the required size.

4. How should the quadrats be placed?

The aim is to remove personal choice as to where the quadrat is placed. "Throwing" a quadrat is not truly random.

We can approach sampling in two different ways: **random** sampling or **systematic** sampling. We will look at each in turn to see how it is carried out.

Random sampling - Ideally every place within the sampling area should have an equal chance of being sampled, each time a sample is taken.

To achieve this, place a tape measure along two sides of the area being studied. Then find random coordinates as follows:

- The length of one side of the quadrat forms the sampling interval. Then divide the length of the plot into these intervals e.g. if you use a 10 x 10 m plot and a 50 x 50 cm quadrat, the intervals will be 0, 0.5, 1.0, 1.5. . . 9.5, 10.
- Write the intervals on pieces of paper and put them into a hat.
- Let each pair of students draw out 2 pieces of paper. (Replace the first piece before taking the second.)
- Each student then finds his or her appropriate position along the tape measure. They turn into the plot at a right angle to the tape and walk into the plot until they meet. This is their sampling position.

Systematic sampling - This is most useful when a pattern in the vegetation is being investigated, for example when looking at the change in abundance of plant species across a pathway.

Lay out a tape measure and place quadrats at regular intervals along the tape measure. Make sure you choose an interval that is small enough to demonstrate any changes taking place. You can even place the quadrats end over end.

5. How many quadrats should be placed?

The sample size depends on how much variability is shown by the plants within the study area. For this reason, when working with younger pupils it is often best to try and avoid areas where plants show obvious clumping.

If the area is fairly uniform ensure that at least 2% of the total area has been sampled by the quadrats. This should give a reasonable size sample.

Example
The plot is 20 m x 20 m (400 m^2)
50 x 50 cm square quadrats are being used, so there are 4 quadrats to the square metre.
It would take 1600 quadrats to cover the whole area.
To cover 2%, we would need 32 quadrats.

If you have a class with 24 pupils divided into 12 pairs, each pair needs to do at least 3 quadrats. You may wish them to do more as part of the learning process, but make sure the grass is not over-trampled!

Dr Anne Bebbington,
Field Studies Council, Juniper Hall

References

Chalmers, N and Parker, P (1989) The OU Project Guide (second edition) Occasional Publication 9 Field Studies Council

Identification fold-out charts are available from the Field Studies Council. A publications list can be obtained from: Field Studies Council Publications, Preston Montford, Shrewsbury SY4 1HW. www.field-studies-council.org Tel: 01743 852140