Plant mineral nutrition in the classroom:

the radish, Raphanus sativus *L is a good plant for such studies*

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Successful mineral nutrition experiments using the cultivated radish, Raphanus sativus L, *can be carried out in two to three weeks in a school laboratory using simple and inexpensive apparatus. The interplant variation is smaller with radish than with some other species. A clear 'crop' is produced and it is easy to record the growth of both the tap root and the leaves. The effects of nitrogen and other nutrients, on growth, are reported here.*

INTRODUCTION

Plant mineral nutrition receives a widespread treatment in syllabuses examined at 16+ and 18+, with increasing emphasis on the problems associated with the application of fertilizer to crops. For example, the programme of study for Key Stage 3 of the science National Curriculum for England and Wales states that pupils should investigate: 'the requirements for photosynthesis in green plants... and the minerals required for healthy growth'. At Key Stage 4 they are required: 'through fieldwork and other investigations, to consider current concerns about human activity... including the use of fertilizers in agriculture...' [1]. At A- and AS-level, there are clear references to mineral nutrition in the various biology syllabuses.

Classroom investigations on plant mineral nutrition require careful preparation and it is often several weeks before any significant results are evident, by which time the class may be studying a completely different topic. Moreover, experiments left in school laboratories for long periods sometimes suffer from accidental interference. Another problem is that pupils may lose interest if there is a long time interval between setting up the material and obtaining results. In order to overcome these problems we tried to use plants which grow quickly. Rapid-cycling *Brassica rapa* (syn *campestris*) L, grows fast and is a useful resource for teaching many aspects of plant science [2], including genetics [3], and seemed to be a good candidate for work on mineral nutrition. However *B rapa* is an outbreeder and even a superficial glance at a sample of the plants, grown under identical conditions, reveals that there is considerable variation in many of the characteristics which might be used to measure growth under different nutrient regimes. When the dry mass of individual plants within various N, P and K treatments was compared, the inter-plant variation was very large (often, the largest plant in each treatment was over 50 times larger than the smallest) so that no statistically significant differences in dry mass were recorded even with 24 replicates per treatment [4]. The variation in dry mass was not normally distributed in each treatment, but was strongly skewed towards the smaller plants. It seems likely that this variation reflects the genetically heterogeneous character of the populations of rapid-cycling *B rapa* currently available [5].

Studies of mineral nutrition are easier using a plant which is genetically more homogeneous than *B rapa* and it was for this reason that the common garden radish, *Raphanus sativus* L was chosen for the study described here. It has several advantages. Genetically homogeneous lines of the radish are produced commercially for the domestic market, and packets of seed are widely available and inexpensive. The plants grow rapidly under continuous illumination from ordinary white fluorescent tubes. Without such illumination, growth is likely to be much less satisfactory. Light banks of fluorescent tubes are widely used by schools and colleges taking part in the Science and Plants for Schools (SAPS) programme in the UK and make possible a wide range of interesting work with plants. Radish plants are compact so that large numbers can be grown in a small space, an important consideration for busy school laboratories. Most important of all, the radish produces a recognizable 'crop' at the end of the investigation and the value of this in engaging the interest of young people in plants, should not be underestimated [6]. In addition, for more sophisticated studies, use can be made of the fact that several different varieties are available enabling comparisons between them to be made. The variety used for this study was Suttons 'Short top forcing' which was chosen because it is described as suitable for sowing at any time of year.

METHODS

The seeds were planted in black film cans filled with a 1:1 mix of silver sand and

Figure 1 Mature radish plants growing in film cans in different nitrogen concentrations

'Perlite'. Film cans can be obtained free of charge from many high street film processors particularly if the request is accompanied by an explanation of the purpose for which the film cans are required and a large bag to put them in. The sand was washed to remove minerals, peat and clay. Sufficient mix for 20 film cans was prepared as follows:

1 Ten film cans of sand (about 100 cm^3) were placed in a polythene bucket.
2 The sand was covered with tap water, thoroughly swirled and allowed to settle.
3 The excess liquid was poured off.
4 This process was repeated until no further colour was washed out of the sand.
5 The sand was rinsed three times with about one litre of distilled water each time.
6 The sand was carefully drained and ten film cans of Perlite (about 100 cm^3) were added. The sand and Perlite were mixed thoroughly.

A heated screwdriver was used to make a hole (about 0.5 cm diameter) in the base of each film can. A small wick of fine capillary matting was poked through the hole before the can was filled with the sand/Perlite mix. The cans were placed on capillary matting supported on the lids of suitable plastic containers depending on the scale of the experiment. Some experiments were carried out with 17 × 17 × 8 cm plastic reservoirs (2 kg margarine containers) with 22 × 16 cm lids (from 4 litre ice cream containers). This system held 2 litres of nutrient solution and could accommodate 16 film cans (Figure 1).

A simpler arrangement is shown in Figure 2 in which groups of 9 film cans (3 rows of 3) were placed over a reservoir made from an 11 × 17 × 6 cm plastic tub (1 kg margarine container). The cans were supported over the reservoir on the lid (13 × 19 cm) which was turned sideways and upside down. This lid overhung at two ends and left a gap at the other two sides for the capillary matting. Two holes (about 0.5 cm diameter) were made in each side of the lid so that the rim did not hold any nutrient solution. A 10 × 23 cm piece of capillary matting was placed over this support and dipped into the reservoir at each side. Each reservoir was filled with about 1.5 litres of nutrient solution.

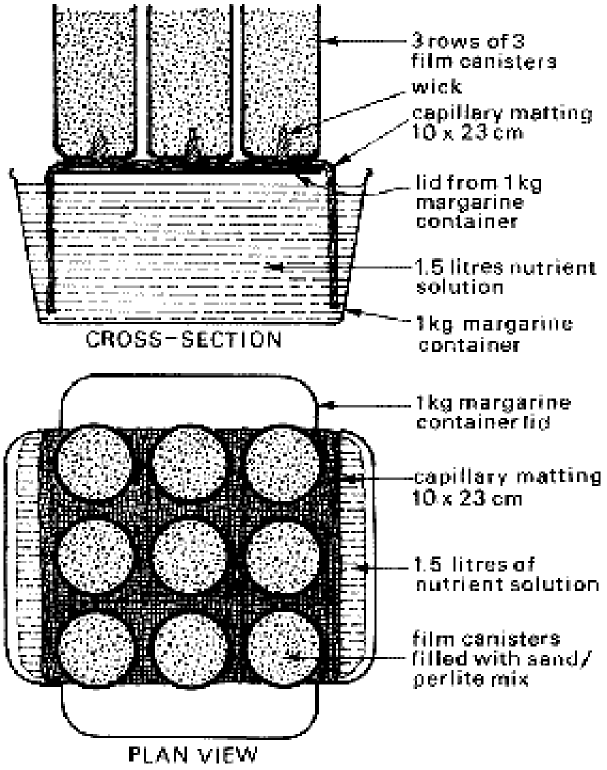

Figure 2 The nutrient culture system

The plants were grown in continuous illumination from a bank of eight white fluorescent tubes, 150 cm (five feet) long, each of 65/80 watts output. The height of the lights was adjusted during the growth of the plants so that they remained about 15 cm above the top leaves.

Table 1 lists the stock solutions used to make up the nutrient solutions. These stock solutions are stable indefinitely if stored in a dark cupboard. Table 2 lists the levels of nitrogen used in an experiment to determine the optimum level of nitrogen and gives details of the volume of stock solutions needed per litre of final nutrient solution.

One seed was planted in each can at a depth of about 1 cm by holding the seed in blunt forceps and forcing it into the growing medium. The lights were left switched off for the first two days of the experiment to allow the seedlings to establish and the

Table 1 The stock solutions used to make up the nutrient solutions

Chemical	g/250cm^3 stock solution	cm^3/2 litre batch of all 6 solutions
Potassium nitrate (KNO_3)	25.25	35
Potassium chloride (KCl)	18.64	25
Sodium dihydrogen phosphate(v) $NaH_2PO_4 \cdot 2H_2O$	39	60
Calcium nitrate ($Ca(NO_3)_2 .4H_2O$)	59	14
Magnesium sulphate ($MgSO_4.7H_2O$)	61.5	48
Calcium chloride ($CaC1_2$)	27.5	106
Micronutrients Solution A		
Copper sulphate ($CuSO_4.5H_2O$)	0.03	
Manganese sulphate ($MnSO_4$)	2 .8	2.4
Zinc sulphate ($ZnSO_4$)	0.36	
Micronutrients Solution B		
Ammonium molybdate ($(NH4)_6Mo_7O_{24}.4H_2O$)	0. 004	2.4
Orthoboric acid (H_3BO_3)	0.325	
Micronutrients Solution C		
Ethylenediaminetetra-ethanoic acid iron(III) monosodium salt ($[CH_2.N(CH_2.COO)_2]_2FeNa$)	1.63	24

wicks to become effective. The reservoirs were topped up with distilled water. In the first week of the experiment no distilled water was required, whilst in the third week, a litre was needed every three days. After 18 days (2 days after Figure 1) the plants were harvested and divided into tops and roots. The dividing line between top and root is clear because the red colour of the root starts where the outer leaf sheath ends. This can be seen in Figure 3.

The film can was easily emptied of its contents and the roots released from the growing medium by immersing the roots and growing medium in a bowl of water. Very few roots had invaded the wick or emerged from the bottom of the can. It was not possible to remove all the Perlite from the fibrous roots. All the thin fibrous roots from the bottom of the red, swollen tap root were therefore cut off and discarded. The samples were air dried at 110 °C for 24 hours

Table 2 The nutrient solutions used in this experiment
(Volume (cm^3) of each stock solution needed for each litre of nutirent solution)

Solution number	**1**	**2**	**3**	**4**	**5**	**6**
Nitrogen level (ppm)	**7**	**14**	**28**	**56**	**112**	**224**
Potassium nitrate	0.5	1.0	2.0	4.0	5.0	5.0
Potassium chloride	4.5	4.0	3.0	1.0	0	0
Sodium dihydrogenphosphate(v)	5.0	5.0	5.0	5.0	5.0	5.0
Calcium nitrate	0	0	0	0	1.5	5.5
Magnesium sulphate	4.0	4.0	4.0	4.0	4.0	4.0
Calcium chloride	10.0	10.0	10.0	10.0	8.5	9.0
Micronutrients Solution A	0.2	0.2	0.2	0.2	0.2	0.2
Micronutrients Solution B	0.2	0.2	0.2	0.2	0.2	0.2
Micronutrients Solution C	2.0	2.0	2.0	2.0	2.0	2.0

Figure 3 The radish tap roots growing in film canisters 16 days after planting

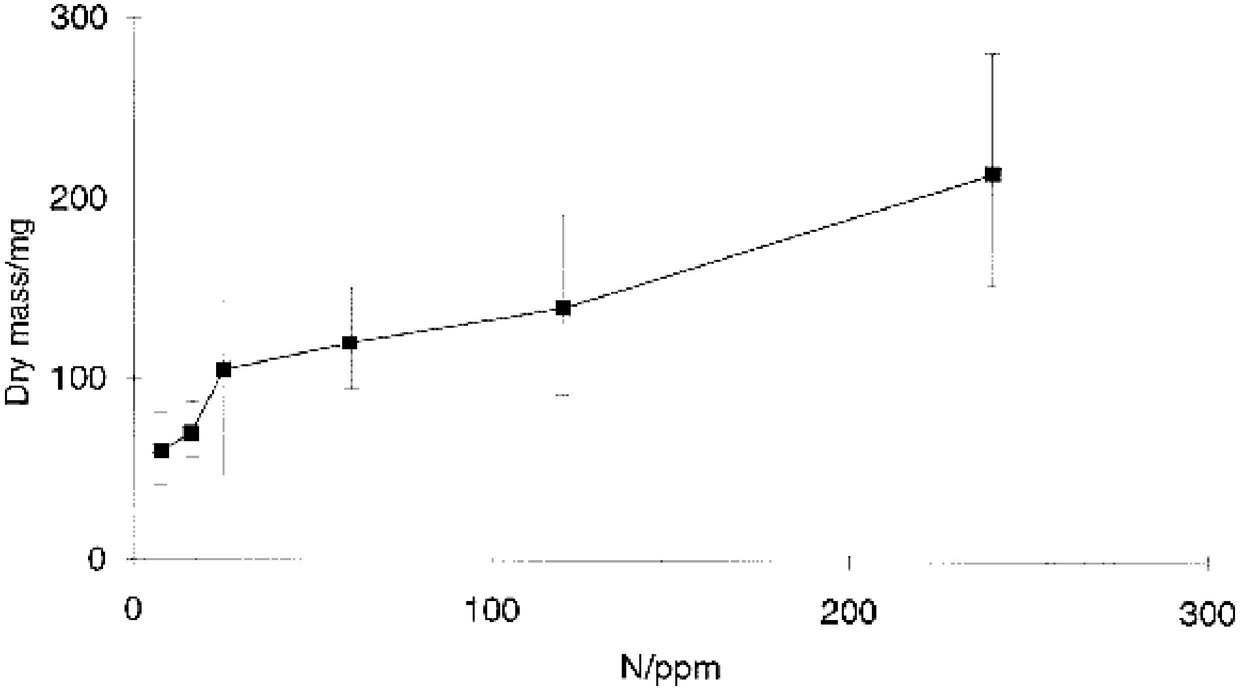

Figure 4 Effect of increased nitrogen on dry mass of tops (leaves etc)
(The vertical bars indicate + and - one standard deviation from the mean)

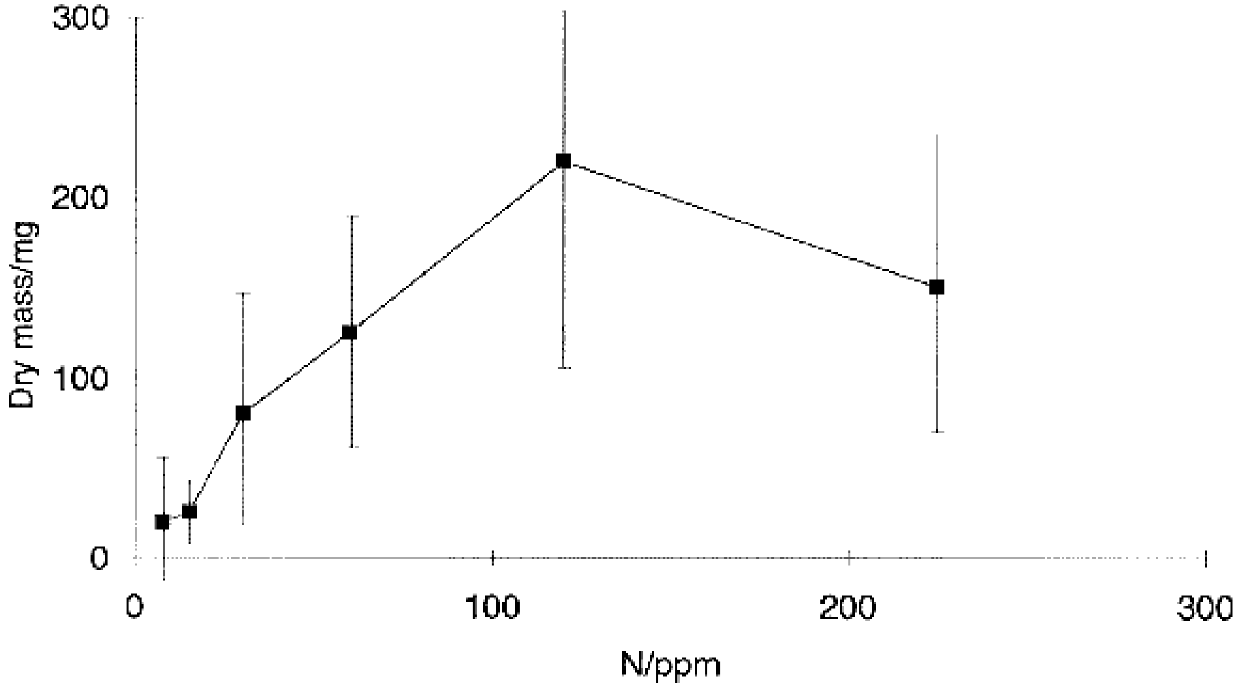

Figure 5 Effect of increased nitrogen on dry mass of roots
(The vertical bars indicate + and - one standard deviation from the mean)

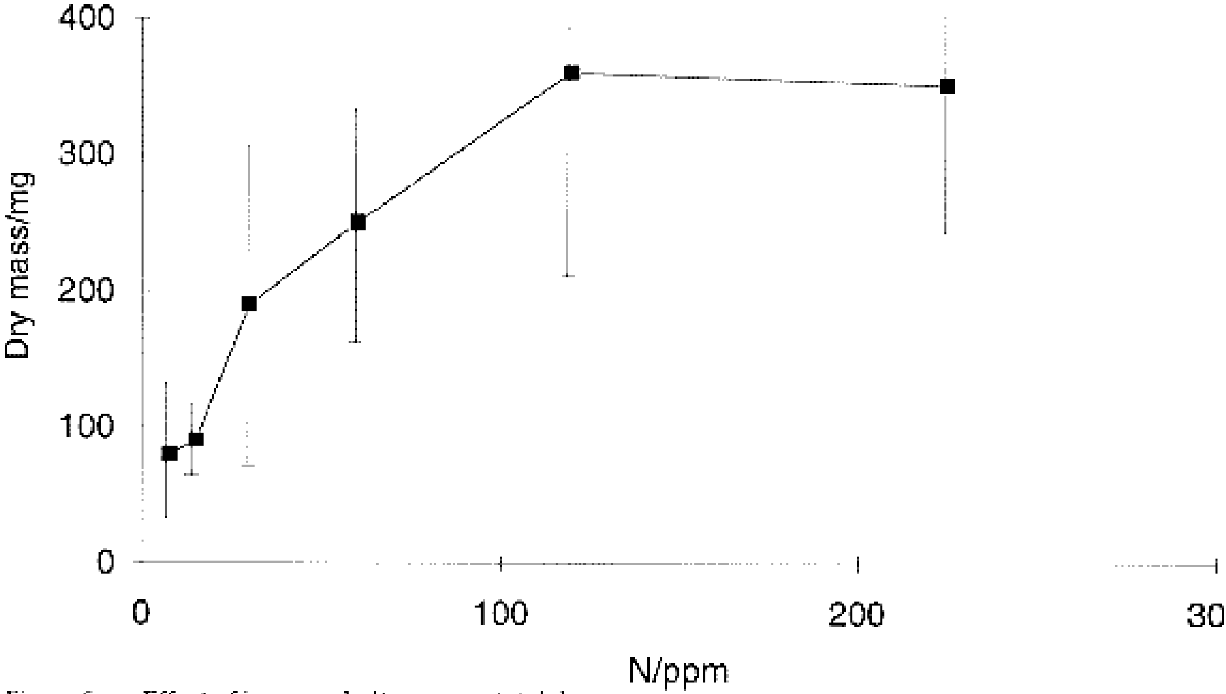

Figure 6 Effect of increased nitrogen on total dry mass
(The vertical bars indicate + and - one standard deviation from the mean)

and their dry mass recorded. 24 hours is more than long enough to dry to constant mass.

RESULTS

A summary of the dry mass data is given in Table 3. Figure 4 shows the effects of nitrogen on the above ground parts of the plant (the tops). The vertical bars on the graphs indicate the extent of ± one Standard Deviation from the mean.

The effect of nitrogen on the dry mass of the roots is shown in Figure 5. This suggests that high levels of nitrogen cause a reduc-

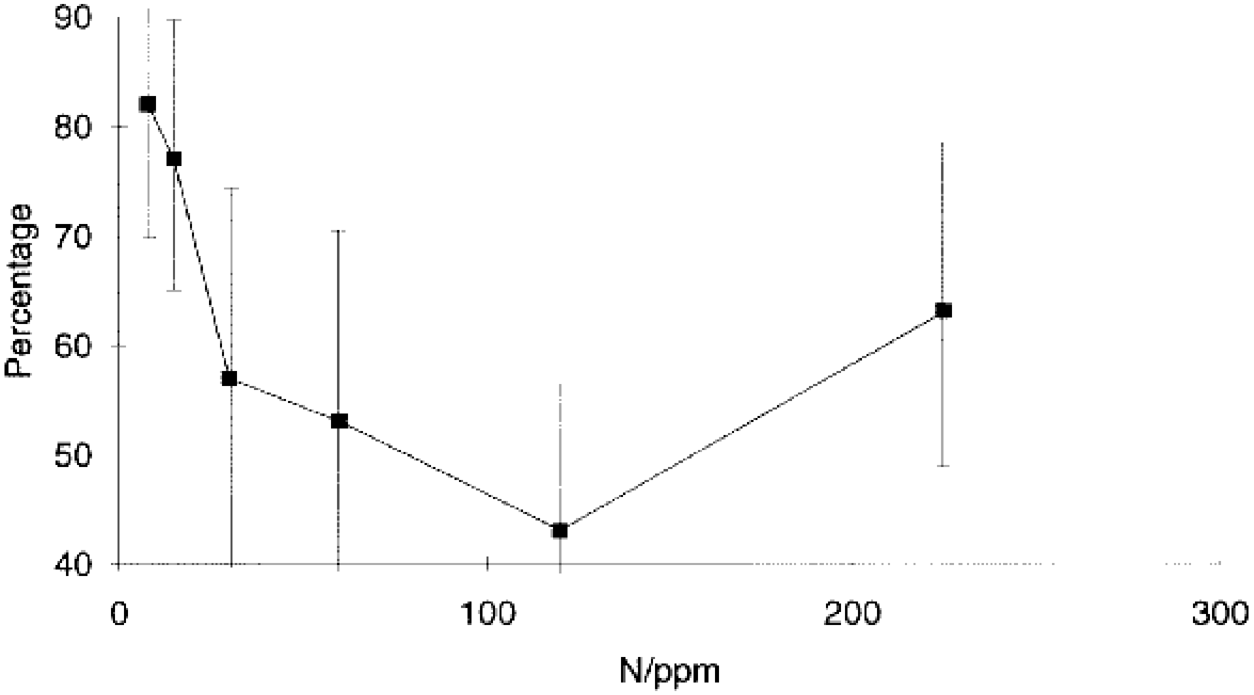

Figure 7 Effect of increased nitrogen on the proportion of dry mass in tops (leaves etc) (The vertical bars indicate + and - one standard deviation from the mean)

tion in yield of radish.

In Figure 6, the effect of nitrogen on the total dry mass is shown. This illustrates a characteristic yield response curve, with diminishing gains from each additional increment of nitrogen.

Nitrogen causes a change in the partition of dry matter between the tap root of the radish and the leaves and this is shown in Figure 7. The smallest roots in proportion to the tops are found in plants grown in 100 ppm. Very small plants (grown in low nitrogen) put almost all their resources into leaf growth.

The statistical analysis of these data is presented in Table 4 where the results of a student's *t*-test are presented for each pair of measurements.

The experiment was repeated, but with fewer plants and a harvest after only 14 days. This produced very similar results and although a full statistical analysis was not performed on this experiment, it would be very suitable as a school demonstration

Table 3 Summary dry mass data/mg

N (ppm)		Tops	Root	Total	% Tops	% Root
7 ppm (16 Plants)	Means	58.2	19.5	77.8	81.5	18.5
	SD	29.2	38.0	59.1	12.0	12.0
14 ppm (17 Plants)	Means	68.0	22.7	90.7	77.3	22.7
	SD	21.7	17.7	32.5	12.3	12.3
28 ppm (16 Plants)	Means	100.5	86.9	187.4	57.3	42.7
	SD	66.7	64.5	115.9	17.3	17.3
56ppm (16 Plants)	Means	118.8	126.2	245.0	52.3	47.7
	SD	35.0	68.6	81.9	18.3	18.3
112ppm (15 Plants)	Means	141.4	217.3	358.7	42.8	57.2
	SD	47.2	117.4	154.8	13.9	13.9
224ppm (15 Plants)	Means	212.1	143.0	355.1	63.1	36.9
	SD	55.4	85.3	126.5	13.7	13.7

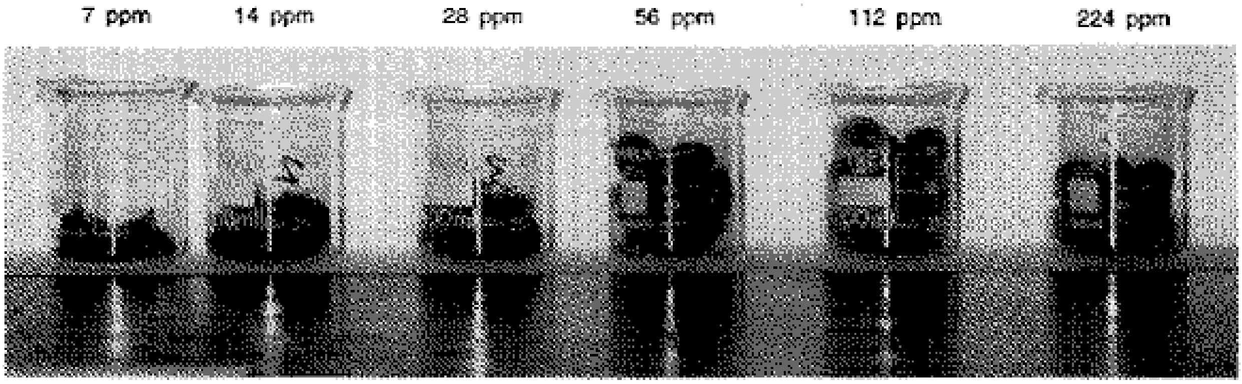

Figure 8 The yield of radish is shown by the height in the beakers

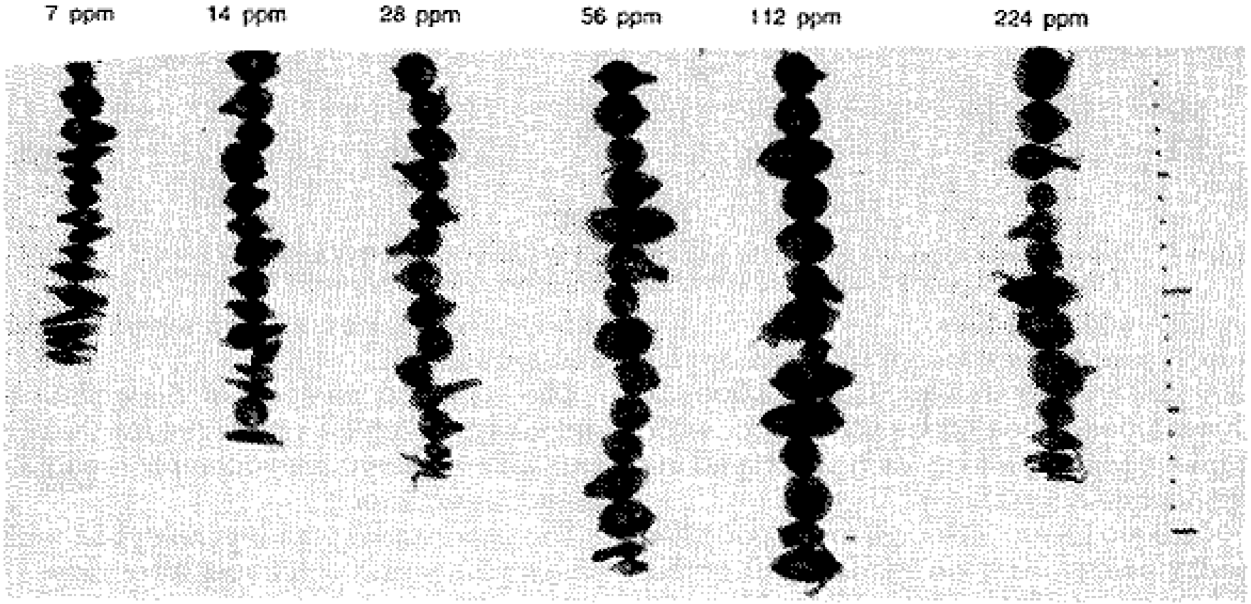

Figure 9 The yield of radish is shown by lining the roots up, side by side, to produce a 'radogram'

of response to nitrogen. The appearance of the plants towards the end of the experiments is shown in Figure 3. It is interesting to notice the occasional yellow plant in treatments which are otherwise healthy in appearance and this may be a suitable topic for further investigation. An interesting way of presenting the results of this experiment is shown in Figures 8 and 9 where the height of the roots in the beakers or better, the sum of the root diameters is shown as a histogram (or 'radogram').

An experiment to investigate the effect of insufficient Ca, Fe, K, Mg, N, P or S was carried out using the same equipment with radish. The reservoirs were filled with Sachs' solutions. Powders to make these solutions can be obtained from laboratory suppliers. The recipe for the solutions is given in Table 5.

The reservoirs were kept topped up with distilled water for 14 days. After this time, it was possible to record clear differences in the dry mass of the plants. This is shown in Table 6 and Figure 10.

Rapid-cycling brassicas have smaller seeds than radish and are therefore likely to pass a smaller reserve of minerals to their seedlings. When grown in sand/Perlite mixture they show clear visual symptoms of mineral deficiency in this experiment after 12 days and spectacular differences after 21 days [4]. However, the variation between plants was so large in a quantitative analysis that only the differences between lack of each nutrient and 'Normal' (complete solution) were statistically significant.

Table 4 Significance of differences
A student's *t*-test was performed on each pair of measurements

Tops	1	2	3	4	5	6
1	X	ns	*	***	***	***
2		X	ns	***	***	***
3			X	ns	ns	***
4				X	ns	***
5					X	***
6						X

Root	1	2	3	4	5	6
1	X	ns	**	***	***	***
2		X	***	***	***	***
3			X	ns	***	*
4				X	*	ns
5					X	ns
6						X

Total	1	2	3	4	5	6
1	X	ns	**	***	***	***
2		X	**	***	***	***
3			X	ns	**	***
4				X	*	**
5					X	ns
6						X

%Root	1	2	3	4	5	6
1	X	ns	***	***	***	***
2		X	***	***	***	**
3			X	ns	*	ns
4				X	ns	ns
5					X	***
6						X

*P=0.05 **P=0.01 ***P=0.001 ns=not significant

DISCUSSION

The apparatus and techniques reported here are cheap and simple and therefore good for use in schools. Furthermore, the experiments can be completed within a two-week period, enabling results of immediate relevance to be obtained. The classic response to nitrogen can be demonstrated by the 'law of diminishing returns' which explains why there is a most profitable level of fertilizer to use. At higher levels of fertilizer, the cost of the additional fertilizer may not be repaid by the extra crop produced. This can be illustrated from the data for Total Dry Mass shown in Table 3.

It is clear that, above 14 ppm, the more nitrogen that is applied, the less additional crop is being obtained per unit of additional nitrogen. There is more radish tissue in response to extra nitrogen at low levels of fertilizer than at high levels. This illustrates that, commercially, there comes a point where it costs as much to buy the extra fertilizer as is returned in extra yield.

There are several possible areas for further investigation:

1 When radishes are grown in nutrient culture (especially in Sachs' solution), occasional plants produce yellow/white leaves. Is this a result of micronutrient deficiency? (Sachs' solutions do not sup-

Table 5 The composition of Sachs' nutrient solutions

Normal solution:	potassium nitrate 0 70g; calcium sulphate 0.25g; calcium phosphate(v) 0.25@; iron(III) chloride 0.005g; magnesium sulphate ().25g; sodium chloride 0.08g; distilled water one litre.
Lacking nitrogen:	substitute potassium chloride 0.52g for potassium nitrate.
Lacking phosphorus:	substitute calcium nitrate 0.16g for calcium phosphate(v).
Lacking sulphur:	substitute calcium chloride 0.16g for calcium sulphate, and magnesium chloride 0.21g for magnesium sulphate.
Lacking potassium:	substitute sodium nitrate 0.59g for potassium nitrate
Lacking magnesium:	substitute potassium sulphate 0.17g for magnesium sulphate.
Lacking calcium:	substitute potassium sulphate 0.20g for calcium sulphate, and sodium phosphate(v) 0.71g for calcium phosphate(v).
Lacking iron:	omit iron(III) chloride

Table 6 The dry mass of radish grown in different Sachs' solutions

		Dry mass/mg				
Treatment	No of plants	Leaves	Roots	Leaves/plant	Roots/plant	Total
Norm	8	1880	1110	235	139	374
-K	10	1880	1390	188	139	327
-Fe	8	1850	770	231	96	328
-N	10	1410	750	141	75	216
-Ca	9	1500	360	167	40	207
-Mg	9	1600	260	178	29	207
-P	9	1670	140	186	16	201
-S	9	1220	460	136	51	187

Table 7 The yield response to added nitrogen

Treatment interval	**Total dry matter/mg**	**Yield for extra nitrogen added**
7-14 ppm	77.8-90.7	1.84 mg/ppm
14-28 ppm	90.7-167.4	6.91 mg/ppm
28-56 ppm	167.4-245.0	2.06 mg/ppm
56-112 ppm	145.0-358.7	2.03 mg/ppm
112-224 ppm	358.7-355.1	-0.03 mg/ppm

ply all the micronutrients.) It would be possible to investigate this by painting solutions containing different micronutrient elements onto the leaves of affected plants. Iron, magnesium and potassium could all be tried both individually or in combination.

2 There may be a difference between radish varieties in their response to nitrogen. Some of the differences might be in qualitative factors such as colour and growth habit, but others will probably be quantitative, eg mass, leaf length etc).

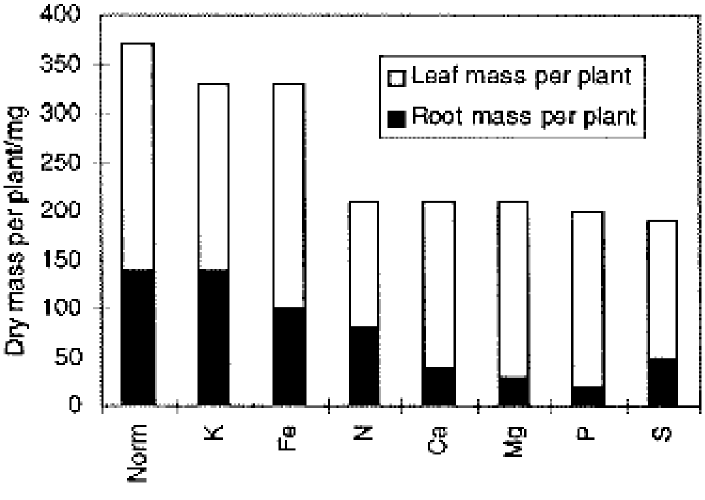

Figure 10 The growth of radish in different Sachs' solutions

3 Only dry mass was recorded in this experiment. However it is likely that there are differences in the density (specific gravity) of the radish tap root. This would affect the eating quality of the crop. Density could be measured by fresh mass weighing and by volume measurement using Archimedes' principle.

4 Radish leaves are simple and few per plant. It would be easy to produce a correlation (either graphically or by calculation) between leaf area and length x breadth. This would enable non-destructive growth analysis of the plants to demonstrate the leaf growth curve, assess when the plants have reached full size and allow calculation of Leaf Area Index (LAI) etc as is now required in certain syllabuses at A-level (eg Cambridge 9260).

5 The sand could be carefully sucked off the tap root without disturbing the fibrous roots so that the growth of the tap root could be monitored by measurement of diameter. How does the growth of the tap root compare with the growth of the tops? There are reports that root crops grow fastest in the higher humidity of nighttime when there is less demand for water by the transpiration stream. This could be checked by comparing measurements taken during the dark period with those taken during the light period when the plants are receiving intermittent illumination.

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