Changes in morphology, proline content, and palatability to Helix aspersa, in Plantago lanceolata in response to transplantation and polluted soil

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CHANGES IN MORPHOLOGY, PROLINE CONTENT, AND PALATABILITY TO *Helix aspersa*, IN *Plantago lanceolata* IN RESPONSE TO TRANSPLANTATION AND POLLUTED SOIL.

by

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Abstract

High concentrations of heavy metals are a cause of stress in plants, many of which respond by accumulating high levels of the amino acid proline. Tolerant and intolerant plants may vary in their response, and this may be reflected in the palatability of their leaves to herbivores. *Plantago lanceolata* plants were collected from four sites showing a range of lead pollution, and were tested for tolerance to lead. Plants from one polluted and one unpolluted site were grown in soils with and without lead for four weeks, and their leaves were offered to *Helix aspersa* in palatability tests. The proline concentration in these plants leaves was determined, and some morphological features were examined.

Plants from each site varied in lead tolerance, and this characteristic showed no clear relationship to the lead concentration of their site of origin. This was considered to be the result of gene flow between plants on small areas of polluted and unpolluted ground.

A possible link between increased palatability and high proline concentration was established. High proline concentration was related to recent transplantation more strongly than to lead concentration in the soil used.

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Introduction

Lead has been mined in the Northern Dales since Roman times. Mining ceased at the end of the ninteenth century but it has left a legacy of patchy heavy metal contamination, ranging from the barely detectable bale hills of the earliest smelters (Barker 1978) to unvegetated spoil heaps recently reworked for barytes. Lead is the main contaminant, although zinc is sometimes associated with it, and in places barytes (J.L.Barker, pers. comm.).

There are many undisturbed spoil heaps, and these are usually sparsely vegetated, with a substrate of fine gravels, loose broken rocks and stones, and a thin layer of topsoil in places (Drewitt 1991). They remain relatively unvegetated for a number of reasons, the most important being a high concentration of lead in the soil, and a scarcity of essential nutrients. They support a characteristic plant community, consisting of species which can tolerate the high concentrations of heavy metals. Some species such as *Minuartia verna, Thlaspi alpestre* and *Botrychium lunaria* are, in the dales, found only on contaminated ground. Others such as *Bellis perennis, Plantago lanceolata, Rumex acetosella* and several grasses occur in plant communities on both contaminated and uncontaminated soil (Drewitt 1991). Baker (1987) classifies plants with the former distribution as metallophytes, and those with the latter as pseudometallophytes. He suggests that some of the

latter may be accidentals, that is, ruderals which appear sporadically on spoil heaps, where they grow with reduced vigour. Plants on spoil heaps must tolerate not only high lead concentrations, but also soil with a low nutrient content, poor water holding capacity, and a tendency to erode (Drewitt 1991). The lead concentration may vary considerably over quite short distances (Cooke and Morrey 1981).

Lead is present in soil in several forms, of which water soluble cations in the soil solution are the immediate source of lead for plant roots. It may also be either bound to or adsorbed onto the surface of colloids in the humus or clay components of the soil, and these forms are partly in equilibrium with the cations in the soil solution. It is also found as precipitated forms such as lead sulphide or carbonate, which are not available to plants (Davies 1990, Alloway 1990).

Plants are more affected by the concentration of available lead than by that of total lead. Total lead concentration may be measured by extraction with powerful solvents such as concentrated nitric acid. The concentration available to plants is less easily measured. Various solvents such as ammonium nitrate, acetic acid and EDTA will extract varying fractions (Ure 1990). These solvents have been developed in agricultural advisory work, and there is little basis for supposing that any of them accurately extract available lead, or will provide data applicable to all plants (Davies *et al*

1987). If the concentration extracted from different soils is positively correlated with the concentration found in plants growing on those soils, then the solvent used is probably extracting the available lead (Hughes *et al* 1980). Investigation with different solvents has shown that for lead, most strong extractants such as nitric acid, EDTA and acetic acid can be used to predict leaf concentration, and thus probably the concentration available to the plant (Davies *et al* 1987).

Some taxa are found on lead contaminated soil, and some are not. The classical view has been that tolerance of lead is genetically determined, and that in some species non-tolerant populations contain a few tolerant individuals, which are capable of colonising spoil heaps, thus building up a tolerant population (Baker 1987). There appear to be some examples of phenotypic tolerance. McNaughton *et al* (1974) found that *Typha latifolia* from a site near a zinc smelter, and from an uncontaminated control site, showed a similar degree of tolerance. Growth was inhibited in both on the contaminated soil, but less than would be expected. It seems that species can differ widely in their threshhold tolerance. Baker *et al* (1986) observed loss of tolerance in *Holcus lanatus* when tolerant clones were grown in unpolluted soil. They suggested that phenotypic adjustments may allow plants to survive in habitats to which they are not well adapted, and that, given time in this way, random mutation may produce genotypes which are well adapted.Thus phenotypic

adaptation enables the plant to be in the right place for natural selection to work on the genotypes if they appear. This is known as the Baldwin effect (Simpson 1953).

However, most tolerance appears to be genetically determined. Crosses between tolerant and intolerant plants in a range of grass species produced a range of phenotypes in the F2 generation, suggesting that several genes are involved (Gartside and McNeilly 1974). There is usually a positive correlation between the amount of lead in the soil and the degree of tolerance to the metal shown by plants growing on the soil (Baker 1987).

In order to measure tolerance, plants are grown in nutrient solution or in soil, and some parameter of growth is measured with and without lead in the growth medium. Increase in either biomass or root length are often used. Root growth in particular is very sensitive to the presence of lead. The measurements may be done sequentially, measuring the same plants with and without lead, or simultaneously, using two sets of plants (Wilkins 1978, Pollard 1980).

The species which do not evolve heavy metal tolerant populations appear to be those with less overall genetic variability. The species chosen for this study, *Plantago lanceolata*, has been shown to be of high genetic variability in such characteristics as reproductive effort (Primark and Antonovics 1982), response to different regimes of carbon dioxide concentration and temperature (Wulf and Alexander 1985), and gene flow

distance (Tonsor 1990). Given this variability, it is not surprising that *P.lanceolata* has evolved lead tolerant ecotypes in both the S.E of U.S.A (Pollard 1980) and in England (Wu and Antonovics 1976). *P. lanceolata* is self-incompatible, and the resulting out-breeding produces phenotypically diverse populations (Ross 1973). Gene flow per generation (pollen and seed mediated) has been measured by Tonsor (1989) at between 0.8m and 1.5m. He suggests that these small distances mean that individuals separated by more than a few metres are not likely to be part of the same "genetic neighbourhood". This suggests that tolerance could evolve on quite small areas of polluted soil, but it must be remembered that many of the polluted areas in the dales are small enough to be similar in size to *P.lanceolata*'s "genetic neighbourhood", so that gene flow across boundaries will influence this process.

The physiological basis for metal tolerance in plants appears to be a suite of adaptations for dealing with the metal when it has been absorbed. Avoidance of the metal, by excluding it from the plant, seems to play only a minor role, as the high metal content of tolerant plants from polluted areas shows (Ernst 1975). Lead enters the apoplast of the root system passively in the soil solution, and must enter the symplast if it is to cross the endodermis. In some plants the endodermis forms a partial barrier, and lead accumulates in the root cortex (Hughes *et al* 1980), but in many plants lead also ascends

to the aerial parts of the plant in the xylem sap. On a cellular level, lead may be accumulated in some cell compartments, principally the vacuoles, thus maintaining others such as mitochondria at a low concentration. Changes in membrane permeability and the production of lead chelating compounds enable this (Baker 1987). There may also be changes in the structure of some enzymes which enable them to function in higher concentrations of lead, as these changes are found in tolerance to other metals (Ernst 1975).

Tolerant *Agrostis capillaris* plants grow less well in unpolluted soil than intolerant plants of the same species (Wilson 1988), providing evidence that there is a "cost" of tolerance, and that high lead concentrations may stress even tolerant plants.

Proline is an amino acid which is frequently found in high concentrations in stressed plants. Water stress almost invariably results in an increase in proline concentration. It is possible that it is acting as an osmoprotectant, but there is evidence that it is not effective in this role (Bhaskaran *et al* 1985). Proline accumulation has also been observed as an effect of low temperature stress (Chu *et al* 1974), and of osmotic stress due to high salinity in the rooting medium (Stewart and Lee 1974). Some ions affect proline accumulation more than others. In osmotic stress varying the solute used to lower the water potential may affect the extent to which proline accumulates (Chu *et al* 1976). An increase in proline concentration has been found in

seedlings grown in media containing lead, compared with seedlings grown in media containing equimolecular concentrations of potassium, and even compared with seedlings grown in media with a lower osmotic potential but without lead. Thus proline accumulation may be part of the stress response to lead in plants (Saradhi *et al*, 1991). Hanley (1990) using *P.lanceolata* has shown that watering plants with a solution of lead nitrate does in some instances produce a rise in proline concentration, although this varies with time. The role of proline in plant stress is not fully understood. It may be part of the plant's response, or it may be a secondary effect, with no benefit to the plant, and for this reason it could be argued that either tolerant or intolerant plants will have higher levels of proline when grown in lead polluted soil.

Stewart and Lee (1974), using halophytes, found that low nitrogen availability in the soil prevented proline accumulation. Stewart (1972) also showed using excised leaves that proline accumulated by leaves in the absence of a supply of carbohydrate is rapidly oxidised, and he suggests that a high concentration cannot be built up in leaves without a supply of carbohydrate. These findings may be relevant to plants grown in the impoverished spoil heap soil.

An accumulation of proline increases the nitrogen content of leaves, and may make them more attractive to herbivores as food. Some invertebrate herbivores have

evolved the ability to discriminate between food sources, and this can be detected by offering a choice of food, and observing the animal's response. Many molluscs are general feeders, taking a wide range of material, but showing preferences when different foods are available. There is evidence that slugs and snails can discriminate between species of higher plants. Choices made by *Agriolimax caruanae* between thirty different plant species and lettuce as a reference material showed that the plant species varied from high acceptability to complete rejection. In another experiment, *Cepea nemoralis* rejected almost 60% of the fifty two species offered (Dirzo 1980, Grime *et al* 1968). These molluscs also distinguish between morphs in some polymorphic species (Cates 1975, Crawford-Sidebotham 1972). Angseesing (1973) observed the response of three slug species to a choice of acyanogenic or cyanogenic *Trifolium repens*, and concluded that two of the species discriminated in favour of the acyanogenic morph, and one did not. It is possible that some species may discriminate in favour of leaves with high proline concentrations, thus obtaining an increased supply of nitrogenous compounds. Lead stressed leaves with a high proline content may contain high levels of lead, and this may too affect the palatability of the leaves.

The aim of this study is to investigate the relationship between lead tolerance in *Plantago lanceolata*, and its palatability to *Helix aspersa*. As a possible link between

these two variables, proline concentrations in *Plantago lanceolata* leaves in various conditions will be measured. *Plantago lanceolata* was chosen as the study species for this investigation because it is reported to be tolerant of lead (Wu and Antonovics 1974) and is abundant in the area, both on and off lead mine spoil heaps. *Helix aspersa* was chosen because it is abundant in the area (though not notably on spoil heaps) and is reported as discriminating between plant morphs in its choice of food material (Bishop and Korn 1969).

2. Methods

Introduction: sites and collection of material.

Several sites in Swaledale, Arkengarthdale and Teesdale which had plant communities typical of lead mining spoil (Drewitt 1991) were examined in April and May 1992.

Table 1. Sites examined

Site	OS Reference	Altitude(m)
Hurst spoil heaps	NZ052025	340
Mould spoil heaps	NY996024	360
Hushes above Grinton mill	SE036963	430
Slei gill spoil heaps	NZ016025	300
Surrender mill	SD991991	350
Bollihope spoil heaps	NZ007349	260
Spoil heap near Newbiggin	NY926273	350

Soil samples were collected from all sites and were analysed for total lead content. *Plantago lanceolata* was chosen for further study because it is well distributed and quite abundant in soils with high concentrations of lead. Plants from Slei gill spoil heaps were selected for palatability tests. A control site was selected at the same altitude, outside the lead mining area, on Barningham moor lane (OS ref NZ085085). A soil sample from this site was analysed. Plants from Slei gill, Newbiggin, Bollihope and Barningham moor were selected for lead tolerance tests. These sites provided a range of lead concentrations.

On May 19th thirty plants of *Plantago lanceolata* were collected from Barningham moor lane, and thirty from Slei gill spoil heaps. Most of these plants appeared to be seedlings, but some pairs were linked by their root system, and were considered to be clones. They were all treated as separate plants. Sufficient soil for 25 76mm pots was collected from the spoil heap.

It was not feasible to collect soil in this quantity from the Barningham site. Garden soil (analysed for lead) was substituted for this. All the soil used for potting was sieved through a 3mm mesh to remove large pebbles and vegetation.

Fifteen plants from the spoil heap were established in soil from the spoil heap, and fifteen in garden soil. Similarly fifteen of the plants from Barningham moor lane were established in spoil heap soil, and fifteen in garden soil.

Ten *Plantago lanceolata* plants were also collected from Bollihope spoil heaps, ten from Moulds spoil heaps, and ten from Newbiggin. These were established in garden soil.

The plants were kept in a shaded unheated greenhouse, standing in waterproof trays. They were watered when necessary with tap water. These plants were used in subsequent experiments.

Soil samples were taken from the rooting zone of all the plants collected.

Table 2. Summary of plants collected, and soils used.

	Slei gill plants	Barningham plants
Slei gill soil	15 (Group A)	15 (Group B)
Garden soil	15 (Group C)	15 (Group D)
Garden soil	Bollihope plants	Newbiggin plants
	10 (Group E)	10 (Group F)

Site descriptions

Slei gill site is part of an extensive area of old lead mine spoil in Arkengarthdale. The vegetation is approximately 2-4cm high, and is grazed by sheep and rabbits. About 30% of the area is bare ground, but there are small pockets of topsoil which are completely vegetated. The plant community includes both metallophytes and pseudometallophytes, and there is a scattered population of *Plantago lanceolata*.

The site at Bollihope is also part of an extensive area of old spoil heap. The vegetation is approximately 2-4cm in height, and is grazed by rabbits and sheep. About 50% of the site is bare ground, and there is less topsoil than at Slei gill site. There is a plant community of pseudometallophytes, and a large population of *Plantago lanceolata*.

The site near Newbiggin in Teesdale is a small area 5m x 5m on a lane verge. It is notable for its population of *Thlaspi arvense*, an indicator species for lead pollution. The site is now level, but it is assumed that there was a spoil heap there in the past. It supports a varied plant community including *Plantago lanceolata,* with a vegetation height of 10-30cm. There was no evidence of grazing.

The site near Barningham is also on a roadside, and is lightly grazed by rabbits. There is a small population of *Plantago lanceolata* in a typical grassland community. The vegetation height is 3-10cm. Both roadside sites are subjected to very light traffic, but it was not considered that this would have raised the lead levels in the soil.

PLATE 1 BARNINGHAM MOOR SITE

PLATE 2 BOLLINGHOPE SPOIL HEAPS SITE

PLATE 3 NEWBIGGIN SITE

PLATE 4 EXAMPLES OF PLANTS FROM FOUR GROUPS

KEY		
	Slei Gill plants in garden soil	Barningham plants in garden soil
	Slei Gill plants in Slei Gill soil	Barningham plants in Slei Gill soil

2.1 Analysis of soil and leaf samples for lead

2.1.1. Collection of samples

Initially soil samples were collected from spoil heaps at Hurst, Moulds, Slei gill, Bollihope, and Newbiggin, and from Grinton mill, Surrender mill, Barningham moor and the garden soil used for potting. One sub-sample from each was analysed for total lead.

Further samples were collected from Bollihope, Slei gill and Newbiggin spoil heaps, Barningham moor, and the garden soil used for potting. These samples were taken from the rooting area of the plants collected. Two sub-samples from each were analysed for total lead, and two for available lead.

Leaves were collected from plants in groups A, B, C and D, and analysed for total lead. 30ml 3M HCl was used with group A plants, and 20ml with groups B, C and D. This allowed thorough mixing of the leaf material. One sub-sample of each group was analysed, as shortage of leaf material prevented replication.

2.1.2. Lead extraction

All samples were dried to constant weight at 850C. Sub-samples of approximately 5g each were accurately weighed out from each sample. To extract 2/3 total lead, a sub-sample was thoroughly mixed with 15ml 3M HCl, and allowed to stand for 30 minutes (P.R.Evans, pers.comm.).

To extract "available" lead, a subsample was thoroughly mixed with 15ml 0.5M EDTA, and allowed to stand for 30 minutes (Ure, A.M. 1990). All the suspensions were filtered through Whatmans no. 1 filter paper.

2.1.3. Lead determination

The filtrates' absorbance at the wave length of lead was measured using a Pye unicam SP9 atomic absorbtion spectrophotometer, after suitable dilution. A calibration curve was plotted using results obtained from the following concentrations of lead nitrate: 1ppm, 2ppm, 3ppm, 4ppm, and 5ppm. The lead concentrations of the filtrates were read from the calibration curve. Total lead concentrations were calculated from the results for samples extracted with 3M HCl. Available lead concentrations were calculated from the results for samples extracted with EDTA.

Means were calculated for total lead and available lead at each site, and the proportion of the total lead which was available was calculated.

2.2. Measurement of lead tolerance

Lead tolerance indices were measured for *Plantago lanceolata* taken from four sites: Slei gill, Newbiggin, Bollihope and Barningham moor. These were chosen to provide a range of values for soil lead concentration. Plants from Moulds spoil heaps were also used in a pilot experiment, as the soil lead concentration was similar to that at Slei gill. The plants were collected and transferred to garden soil for use four weeks later.

The lead tolerance index was calculated as

$$\frac{\text{Root increase during three days with lead}}{\text{Root increase during three days without lead}}$$

This index is specific to the concentration of lead used to obtain it (Wilkins,D.A.1978). The plants were kept at a temperature of 25^0C and a regime of 16 hours light and 8 hours of darkness while the index was measured.

2.2.1. Pilot experiment

A pilot experiment was carried out to improve details of the method, and to ascertain the concentration of lead needed to distinguish tolerant *Plantago lanceolata* plants from intolerant ones.

Five plants from Moulds spoil heap were transferred from soil in pots to water culture. *Plantago lanceolata* roots consist of a black tap root (which

had been cut short) with many white or brown branching roots.These roots were trimmed so that they could be placed in open ended boiling tubes, and dead leaf bases at the base of the rosette were removed, so that a sharp junction between the dark tap root and the pale leaf bases could be seen. The plants were supported in boiling tubes with pieces of plastic foam, and the tubes were suspended in 3.5 l of a solution of calcium nitrate (1g l^{-1}) (Wilkins,D.A.1978). The length of the longest root on each plant was recorded every day. These lengths were measured from the junction between the leaf bases and the root to the root tip, after gently straightening the root. When growth was well established (after 9 days) lead nitrate solution was added to give a concentration of 25 ppm of lead (Wilkins 1977). Root measurements were continued for a further 5 days. The solutions were renewed every 7 days. A graph of root length against time was drawn, and as a result of these observations it was decided that the three longest roots would be measured, and that further experiment was needed with different concentrations of lead before a tolerance index could be calculated.

2.2.2. Experiment to determine the optimum concentration of lead for use in estimating tolerance indices.

Five *Plantago lanceolata* plants from each of the four sites were transferred to water culture, using the same technique as above. Each group was in a separate container. A further five plants from Barningham moor were transferred to two containers. One of these (containing three plants) was aerated daily for 15 minutes. The other (containing two plants) was not. These two containers were used to observe any effects on growth of changes in the concentration of dissolved oxygen in the solutions. No lead was added to them, and therefore the two plants which were not aerated also served as a parallel control.

The three longest roots of each plant were measured daily. In most cases, several days elapsed before growth resumed. At least four days after growth had restarted in all the plants in a container, 2.5ppm of lead was added as lead nitrate. Not less than four days later, the lead concentration was increased to 5ppm and finally, after at least another four days, to 10ppm for plants from some of the sites. Root measurements were continued on a daily basis, but some days were missed, and these results were interpolated where necessary for the T.I. calculations.. Two groups of Bollihope plants were used in order to obtain results more quickly.

Table 3. Days on which lead concentrations were changed. (measurements started on day 1).

Lead conc.	Barn	Boll1	Boll2	Slei	Newb
2.5ppm	8	12			7
5.0ppm	12		7	8	10
10.0ppm	18			17	14

Roots which grew out of the end of the boiling tube were trimmed. In some cases roots broke off but usually others could be substituted, and the sequence of increases maintained.

Mean root lengths for each plant for each day were calculated. Provisional lead tolerance indices were calculated for each lead concentration, using the growth rate before any lead addition, and the growth rates after each concentration had been reached. This was not completely satisfactory, as the two measurements were separated in time by varying amounts, but it enabled the selection of a concentration of 2.5ppm for a definitive determination.

2.2.3. Determination of tolerance indices.

Plants from the four sites were re-established in calcium nitrate solution. Some of these plants were those used in previous experiments, others were new ones, transferred from pots. Three plants from Bollihope and five from each of the other sites were used. Two further plants from Barningham were established in a separate container of calcium nitrate solution. No lead nitrate was added to this

container, so that these plants were a parallel control.

When root growth was well established, and had been measured for four days, 2.5ppm of lead (as lead nitrate) was added to the other containers, and root measurements continued for a further four days. The solution was changed every four days. Tolerance indices were calculated from these results, and visible changes in the roots were recorded. A control tolerance index, using measurements on the same days, was calculated for the control plants.

Composition of the control solution.

In measuring T.I.s in this way several choices must be made. The control solution may be a complete culture solution such as Hoagland's solution (Hewitt 1952). This gives rise to a problem with the treatment solution because of the insolubility of many lead salts, particularly lead phosphate (Wilkins 1977). Distilled water may be used, as the plants will only be grown in it for a short time. However this is unrealistic in ecological terms. Furthermore there is evidence for interactions between lead and other ions in their effects on the plants, notably a reduction in the toxicity of lead when calcium is added. Using a control solution containing calcium nitrate allows higher concentrations of lead to be used, and the exact adjustment of the lead concentration is then less

critical (Wilkins 1957). In these experiments 1g l^{-1} calcium nitrate was used as the control solution.

Timing of changes of solution

It is possible to measure T.I.s using either sequential or parallel controls. If using the former, root elongation is measured over 3 days in the control solution, and the plants are then transferred to the treatment solution, and root elongation is measured for a further 3 days. A T.I. can then be calculated for each plant. This assumes that the growth rate would have remained constant if the plants had remained in the control solution. However, if all the conditions which affect the growth rate are constant for all the plants being measured, comparative studies are possible even if the growth rate changes with time. An investigation with parallel controls uses two sets of plants, one in the control solution, and one in the treatment solution. This assumes that the control and treatment plants have similar growth rates, which can be compared (Wilkins 77). In the present experiment the plants were not clones, and as *P. lanceolata* is a very variable species it seemed likely that plants from the same site would vary. For this reason the sequential method was chosen, and a parallel control for Barningham plants was set up, in order to observe changes in the growth rate with time.

2.3. Proline Determination

The free proline content of leaves from four plants in each of groups A, B, C and five plants in group D was determined using a method based on those described by Bates et al (1973), Chinard (1952) and Troll and Lindsey (1954).

Acid ninhydrin was prepared by adding 1.25g of ninhydrin to 30ml glacial acetic acid and 20ml 6M phosphoric acid and warming the mixture to 80^{0}C in a water bath until the ninhydrin was completely dissolved. Fresh ninhydrin solution was prepared for each determination, although it is stable for 24 hours (Troll and Lindsley 1954).

Approximately 0.5g of plant material was ground up in a pestle and mortar with a very small quantity of purified acid-washed sand until the mixture was homogenous. 25ml of 3% sulphosalicylic acid was added, and the mixture ground for one minute.

Where insufficient plant material was available, the weight used, and the volume of sulphosalicylic acid used, were reduced proportionately. Sulphosalicylic acid is an effective protein precipitant in aqueous solution, thus removing proteins which might be hydrolysed, liberating proline, at a later stage of the procedure. It is colourless, and it does not

interfere with the acid ninhydrin reaction (Bates et al 1972).

The mixture was filtered through Whatman No.1 filter paper. Three replicate volumes of 2ml of filtrate were measured out, and each was shaken vigorously with approximately one-tenth its weight of Amberlite (Permutit resin). This removes lysine and ornithine quantitatively from the solution, but does not remove proline (Troll and Lindsey 1954).

2ml of glacial acetic acid, and 2ml of acid ninhydrin were added to each 2ml of filtrate, and the mixture was shaken. The test tubes were covered, and placed in a water bath at 80^{0}C for one hour. At the end of this time, they were removed and allowed to cool to room temperature.

At a pH of approximately 1.0, a pink water-insoluble reaction product is formed by proline with ninhydrin. The greater the proline concentration, the more intense is the pink colour. Lysine forms a black product, and ornithine a red one, hence the need to use Amberlite to remove these amino acids. No significant amounts of colour are formed with most other amino acids at a pH near 1.0 (Chinard 1952).

4ml of the reaction mixture was added to 4ml of histoclear. Histoclear acts in the same way as benzene or toluene (used by Chinard 1952) (J.A.Pearson

personal communication). The mixture was shaken vigorously with a test tube stirrer for 20s, and allowed to settle. The pink proline-ninhydrin product is extracted by the histoclear, which forms the upper layer. This was aspirated from the aqueous layer, and its absorbance read at 560nm, using the Pye unicam spectrophotometer, and using histoclear as a blank. The proline concentration of the reaction mixture was then read off from a calibration curve.

The calibration curve was plotted using results obtained from the following solutions of hydroxyproline free L-proline: 5μg cm^{-3}, 10μg cm^{-3}, 25μg cm^{-3}, 50μg cm^{-3}, 100μg cm^{-3}, and 200μg cm^{-3}.

The proline concentrations of the leaves were then calculated using the following formula (Chinard 1952):

$$\frac{(\mu g \text{ proline/ml} \times \text{ml histoclear})/115.5}{\text{g sample}/A}$$

$$= \mu\text{moles proline/g of plant material.}$$

$$A = \frac{\text{vol. of sulphosalicylic acid used}}{\text{vol. of filtrate used}}$$

The factor 115.5 in the equation is the A.W of lead, and an extraction coefficient, which is the same for toluene, benzene and histoclear.

Mean proline concentration for leaves from each group was calculated, and the results were analysed using t-tests.

2.4. Morphological studies

Ten plants were selected randomly from each of groups A, B, C and D four weeks after they had been potted in the soils described.

2.4.1. Leaf size and proportion

The greatest width and the length from base to tip was measured for the longest leaf on each plant. The ratio of length to width was calculated.

2.4.2. Number of leaves with and without petioles

Older *Plantago lanceolata* leaves have petioles, which merge with the leaf without a sharp junction. Younger leaves do not have a petiole. The number of leaves with and without petioles was recorded for each plant except, by an oversight, those in group A.

2.4.3. Specific leaf weight (SLW)

Weight mm^{-2} was calculated for the leaves used in palatability tests, and this data was used to calculate the mean weight mm^{-2} for each of the four groups. This measurement is the specific leaf weight of the leaf (Teramura 1983).

Analysis of variance was carried out on all these results, and t-tests were done where appropriate.

2.5. Palatability studies

Twenty fully grown *Helix aspersa* were collected and kept in plastic containers. They were fed on lettuce, and enamel paint was used to number them on their shells from one to twenty.

2.5.1. Pilot experiments

In order to determine the best conditions for palatability tests, a pilot experiment was undertaken to answer the following questions:

1. How long should the snails be starved before each test?
2. How long should they be left with the test material?
3. At what temperature should they be kept?
4. How can the test material be maintained in a suitable condition?

Plants from the Science site at Durham were used for the pilot study, as the experimental plants were still small. Snails were starved for one, two or three days before being used. They were placed singly in lidded sandwich boxes, with a piece of damp cotton wool. Pieces of *Plantago lanceolata* leaf with an area of 300mm^2 were weighed, and one was added to each box. Paper clips were attached to some of the leaves, to see whether this was a suitable method for distinguishing the leaves in palatability trials.

Two boxes each containing a snail, a piece of leaf and damp cotton wool, and one containing only a piece of leaf and damp cotton wool were placed in each of three temperatures 15^{0}C, 20^{0}C and 25^{0} C. The weight and area of all the leaf pieces was recorded each day for three days. Further pieces of leaf were offered if the first was completely eaten.

It was apparent that the turgidity of the leaf material varied considerably. As a results of this observation, an investigation into the conditions needed to keep it constant was undertaken. Ten boxes were set up at 15^{0}C. Each contained 1.7g of cotton wool in a shallow open dish. Volumes of water between 20cm^{3} and 40cm^{3} were added to the cotton wool, and 300mm^{2} pieces of leaf were weighed and placed in each box. After one day, the leaves were reweighed, and the changes in weight calculated as a % of the original weight. Full details of these experiments are given in the results section, but as a result of these investigations, the palatability tests were carried out at 15^{0}C, and the snails were starved for one day and left with the leaves for one day. 30cm^{3} of water was added to 1.7g of cotton wool in an open container in each box.

2.5.2. Palatability tests

Ten plants from each of groups A,B,C and D were selected, and the pots were numbered 1 - 10. Ten snails were used in each test. These were either nos 1-10 or nos 11-20. Before a test they were starved for 24 hours.

Eight palatability tests were carried out. In tests 1 to 6, two leaves from different plant groups were offered to each snail. All possible pairs of plant groups were tested in this way. As a control, in tests 7 and 8 each snail was offered two longitudinally separated halves of the same leaf with the midrib removed. Only group D plants were used in these tests.

Table 4. Summary of Palatability tests

Test group	Plant groups	snail
1	A:B	11-20
2	A:C	1-10
3	A:D	11-20
4	B:C	11-20
5	B:D	1-10
6	C:D	1-10
7	D:D	1-10
8	D:D	11-20

Ten sandwich boxes (no.1-10) were set up, with 1.7g of cotton wool and 30cm^3 of water in an open container in each. Each box contained a leaf or part of a leaf from a plant from each of the two groups

being tested. The plants were allocated to the boxes randomly in each test.

The leaves were weighed, and their areas were recorded by drawing round them on graph paper with mm divisions. The weight mm^{-2} was calculated for each leaf. Leaf pieces of approximately equal area were used, but cut edges were kept similar in pairs of leaves as far as possible, as it had been observed that the snails more frequently ate from a cut edge. Coloured paper clips were attached to the leaves, so that the different groups could be identified. One snail was placed in each box, with two pieces of leaf, and the boxes were kept at 15°C for one day. The area of each leaf was again recorded by drawing round it, and the area eaten was measured from this and the previous drawing. The snails were fed on lettuce for two days before being used again.

2.5.3. Treatment of results

The initial measurements of fresh weight and area were used to calculate the weight mm^{-2} for each leaf. This and the area eaten by each snail were used to calculate the fresh weight of each leaf eaten by each snail in each test.

The amount eaten could be measured as weight or area, but changes in water content of the leaves affect the weight, and area measurements ignore variations in leaf thickness. In the present experiment, the weight mm^{-2} of the freshly picked leaves was calculated, the area eaten was measured, and the weight of fresh material eaten calculated from these measurements. Records of weights where a snail had eaten nothing from either leaf, and where a snail had eaten all of both leaves, were not included in the results. The weights were used to calculate palatability indices for each group of plants.

$$\text{Palatability index (P.I)} = \frac{\text{Wt of leaf eaten}}{\text{Weight of both leaves eaten}}$$

Each plant group in turn was considered as the reference material, and the P.I.s of the other three groups when tested against it were compared using analysis of variance and t-tests where appropriate. e.g, the P.I.s of leaves from plant groups A, B, and C when tested against leaves from group D were compared. The P.I.s of pairs of leaf strips from group D were also compared.

3.1. Results of analysis of soil and leaf samples for lead

Table 3.1.i. in the appendix holds the full results.

All these results are calculated as ppm lead for dry weights of soil and leaves.

Table 5 shows the total lead concentrations in soil samples from the 8 sites initially examined, and from the garden soil used in potting.

Table 5. Total lead concentrations on eight sites

Site	Total lead(ppm)
Grinton mill	14089.60
Surrender mill	9868.99
Moulds spoil heap	10146.12
Slei gill spoil heap	9583.83
Hurst spoil heaps	9737.98
Newbiggin spoil heap	3698.86
Bollihope spoil heap	1034.55
Barningham moor	199.12
Garden	427.69

The four sites selected to provide a range of total lead concentrations for further study were Slei gill, Newbiggin, Bollihope and Barningham.

Table 6 shows the mean total concentration of lead, the mean available concentration of lead, and the fraction of the total lead that is available for soil from these four sites, and for the garden soil that was used for potting.

Table 6. Mean lead concentrations on five sites

Site	Total lead (ppm)	Available lead (ppm)	Fraction available
Slei	8,486	4,165	0.49
Boll	760	292	0.38
Newb	3,816	2,058	0.54
Barn	163	63	0.39
Gard	463	168	0.36

The mean concentration of total lead varied from 8,486 ppm at Slei gill to 163 ppm at Barningham, and the mean concentration of available lead varied from 4165 ppm at Slei gill to 63 ppm at Barningham. The fraction of the total lead which is available also varied from site to site, although there was some tendency for higher total lead concentrations to give a higher available fraction. Table 7 shows the concentration of lead in leaves from plants in groups A, B, C and D.

Table 7. Lead concentration in leaves in ppm.

A	Slei plant Slei soil	46.10
B	Barn plant Slei soil	34.43
C	Slei plant gar.soil	50.70
D	Barn plant gar. soil	25.54

There was only sufficient plant material to provide one leaf sample from each group for analysis.

3.2. Results of lead tolerance studies.

3.2.1. Pilot experiment

Tolerance index (T.I.) =

$$\frac{\text{Increase in root length in 3 days in treatment solution}}{\text{Increase in root length in 3 days in control solution}}$$

A T.I. of 1 would indicate that growth had remained the same when the plant was transferred to the treatment solution, and that the plant was tolerant of that solution. A T.I. of 0 would indicate that growth stopped when the plant was transferred, and that the plant was intolerant of the solution. Intermediate T.I. values indicate degrees of tolerance.

The tolerance index for plants from Moulds spoil heap is shown in table 8.

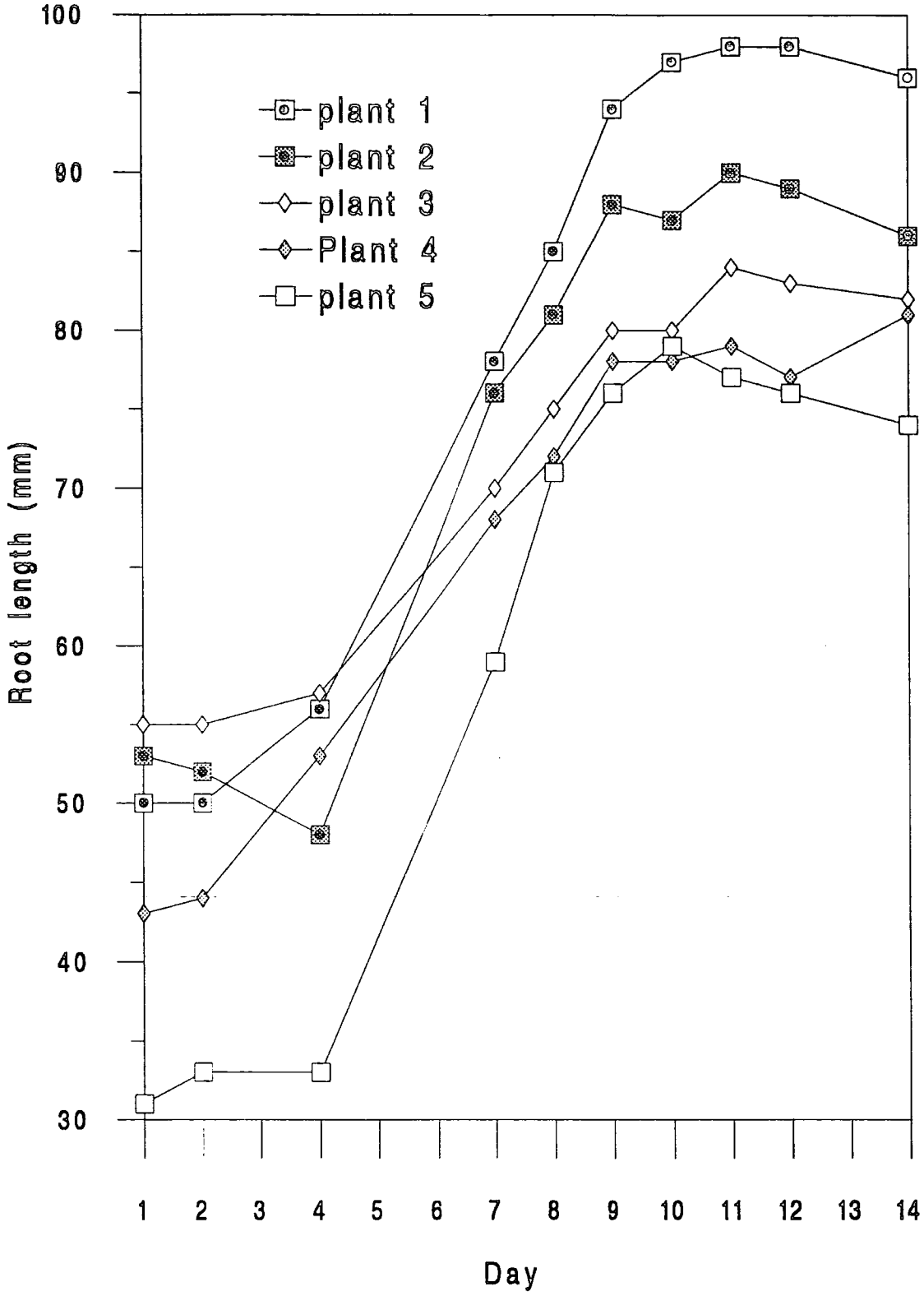
Table 8. Mean tolerance index for plants from Moulds

Mean	n	SEM
0.08	5	0.045

Fig. 1 is a line graph showing the lengths of the longest roots of these five plants on 14 days. It shows that in all the plants, root elongation slowed or stopped on day 9 when 25ppm lead was added. Root lengths, and T.I.s for each plant are shown in table 3.2.1.i in the appendix.

These plants come from a spoil heap where the total lead content of the soil is 10,146ppm. In water culture

Fig 1. Root growth in five plants from Moulds 25ppm lead added day 9



a concentration of 25ppm almost completely stopped root growth. The plants are much more sensitive to lead in water culture than when growing in soil, where interactions with other ions result in a decreased sensitivity to lead.

The T.I. is specific to the concentration of lead used in the treatment solution. A concentration must be found which separates the T.I.s of tolerant and intolerant plants, allowing the most tolerant to grow well, and preventing the least tolerant from growing (Wilkins 1978). In this investgation, 25ppm lead (used by Wilkins (1978) studying *Festuca ovina*) almost stopped the growth of *Plantago lanceolata* plants which were expected to be tolerant. Wu and Antonovics (1976) used 15.6ppm, and Pollard (1980) used 2.5, 5.0, 7.5, and 10.0ppm in distilled water (no calcium) when investigating lead tolerance in *P.lanceolata*. As a result of these observations it was decided to investigate the effects of treatment solutions of 2.5, 5.0 and 10.0ppm on plants from a range of sites.

Elongation of the longest root may not be an accurate measure of root growth. If the longest root is not always the same one, growth will be under estimated. In a plant with many roots, growth in each might be slower than in a plant with few. This is not important when

using sequential controls. Growth may be concentrated in the shorter roots, thus leading to an underestimation. For these reasons it was decided to measure the three longest roots and calculate the mean as a measure of root growth.

3.2.2. Optimum concentration of lead for T.I. estimation.

1. Control plants

The aerated roots grew faster than the roots which were not aerated. Mean root elongation over 5 days in the two groups is shown in table 9

Table 9. Mean daily root elongation in control plants

Treatment	Mean inc.(mm)	n
Aeration	2.99	3
No aeration	0.93	2

Table 3.2.2.i. in the appendix shows the mean root elongation of individual plants.

Aeration of the culture medium increased root elongation considerably. However owing to the practical difficulty of aerating a number of solutions simultaneously it was decided to change the solutions in the final experiment on the first day of both 3 day periods of measurement, to ensure adequate oxygenation of the water during the growing period.

2. Lead treatments

Mean T.I.s for plants from four sites, measured using three concentrations of lead, are shown in table 10.

Table 10. Mean T.I.s for plants in three concentrations of lead

Site	Lead(ppm)	Mean T.I.	n	SEM
Barningham	2.5	0.86	4	0.145
	5.0	0.48	4	0.169
	10.0	0.07	4	0.039
Bollihope	2.5	0.28	5	0.116
	5.0	0.14	4	0.077
Newbiggin	2.5	0.49	5	0.122
	5.0	0.16	5	0.054
	10.0	0.03	5	0.020
Slei gill	5.0	0.19	5	0.086
	10.0	0.06	5	0.028

Mean root growth and T.I.s for each plant from these sites and in these lead concentrations are shown in table 3.2.2.ii in the appendix.

In some cases, roots shrank in the treatment solution. This gave rise to a negative T.I. Lower negative values for T.I. cannot be interpreted as indicating a lower tolerance of lead. For this reason mean T.I.s were calculated from mean root lengths.

The plants on any one site vary considerably in their tolerance, reflecting the facts that they are genotypically distinct, and that *P.lanceolata* is a very variable species.

At 5.0ppm, the plants from Barninghan moor have a higher mean T.I than those from Slei gill. This is unexpected, as Slei gill is heavily contaminated, and Barninghan moor has a low concentration of lead. This result may well be due to the small size of the samples, and the individual variation. These results were not analysed, as the comparison will be repeated in the final determination.

The concentration chosen for the final T.I. determinations should be one which produces significant differences between sites. The results were analysed to determine this. The results are shown in table 11.

Table 11. Results of Analysis for effects of various lead concentrations

ANOVA and t-tests for results with 2.5ppm lead.

	df	M	F	
Totals	13			
Sites	2	0.36	4.79	P < 0.05
Residuals		11	0.08	

T-tests

	Newb	Barn
Boll	P>0.1	P<0.05
Newb	-	P>0.05

ANOVA for results with 5.0ppm lead

	df	M	F	
Totals	17			
Sites	3	0.09	2.24	P > 0.05
Residuals		14	0.04	

ANOVA for results with 10.0ppm lead.

	df	M	F	
Totals	13			
Sites	2	0.00	0.39	P > 0.6
Residuals		11	0.00	

At 2.5ppm, only plants from Bollihope and Barningham are significantly different in T.I. Neither 5.0ppm nor 10.0ppm distinguish significantly between T.I.s for plants from any of the sites. Some sites' plants were not treated with all concentrations.

As a result of these observations, a concentration of 2.5ppm was selected for the final determinations of T.I.

3.2.3. Final determination of Tolerance Indices

A T.I. was calculated for the control plants in the same way as those calculated for the treated plants. Details of all T.I.s are shown in table 12.

Table 12. T.I.s measured in final determination

Site	Mean T.I.	n	SEM
Barningham	0.26	5	0.093
Bollihope	0.06	3	0.035
Newbiggin	0.34	5	0.156
Slei gill	0.23	5	0.130
Control	0.46	2	

Results of ANOVA for T.I.s from four sites

	df	M	F	
Total	17			
Sites	3	0.05	0.72	P > 0.5
Residuals		14	0.07	

No significant difference was detected between the T.I.s for plants from the four sites, possibly due to the small sample size.

The T.I. calculated for the control was less than 1, showing that a decrease in growth rate of the control plants occurred during the determination. The T.I.s for the treated plants were all less than that for the control plants, showing that the treatment had further decreased growth.

Fig. 2 is a graph showing mean root elongation, in both treated and control plants. It shows that in the control plant the decrease in growth rate was uniform throughout the nine days, whereas the treated plants' growth rate decreased abruptly after the addition of lead on day 5.

The relationship between T.I. and available lead at each site is shown in the graph in Fig.3. The control T.I. is also shown, but cannot be compared directly with the others, as it has no value for the x axis. The highest T.I.s are found in plants from Newbiggin, and the lowest in plants from Bollihope. These two sites have intermediate levels of available lead. Slei gill and Barningham have similar intermediate values for T.I. However, Slei gill has the highest concentration of available lead and Barningham the lowest, so that no relationship has been established between the concentration of available lead at a site, and the lead tolerance of *P.lanceolata* plants growing there. Consideration of all the results for T.I determinations shows that on any one site T.I.s for *P.lanceolata* may vary considerably.

Root lengths, and T.I.s for individual plants in the final determination are shown in table 3.2.3i in the appendix.

Fig 2.Mean root lengths of plants from four sites, and control plants, for nine days. 2.5ppm lead added day 5. No lead added to control.

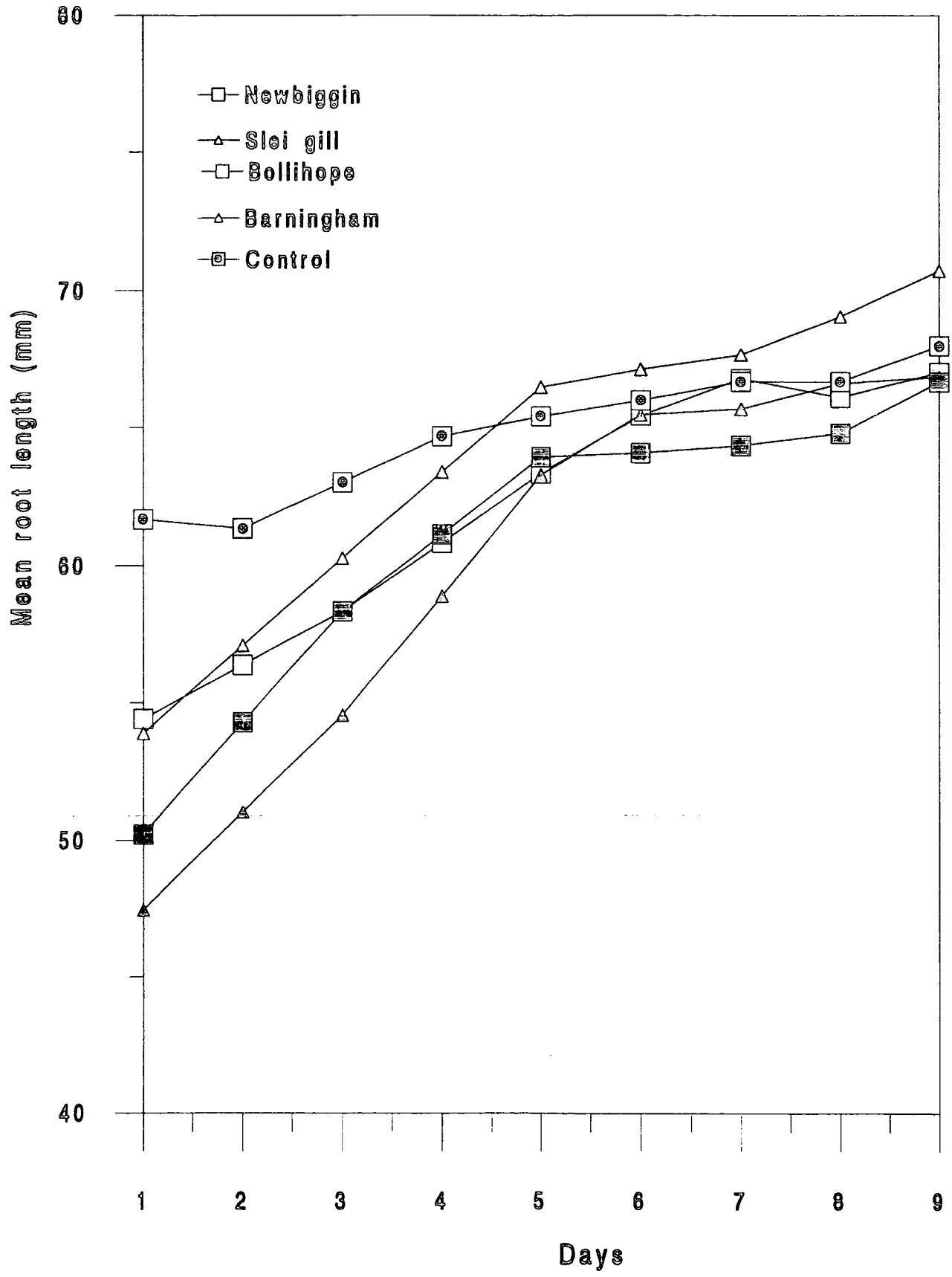
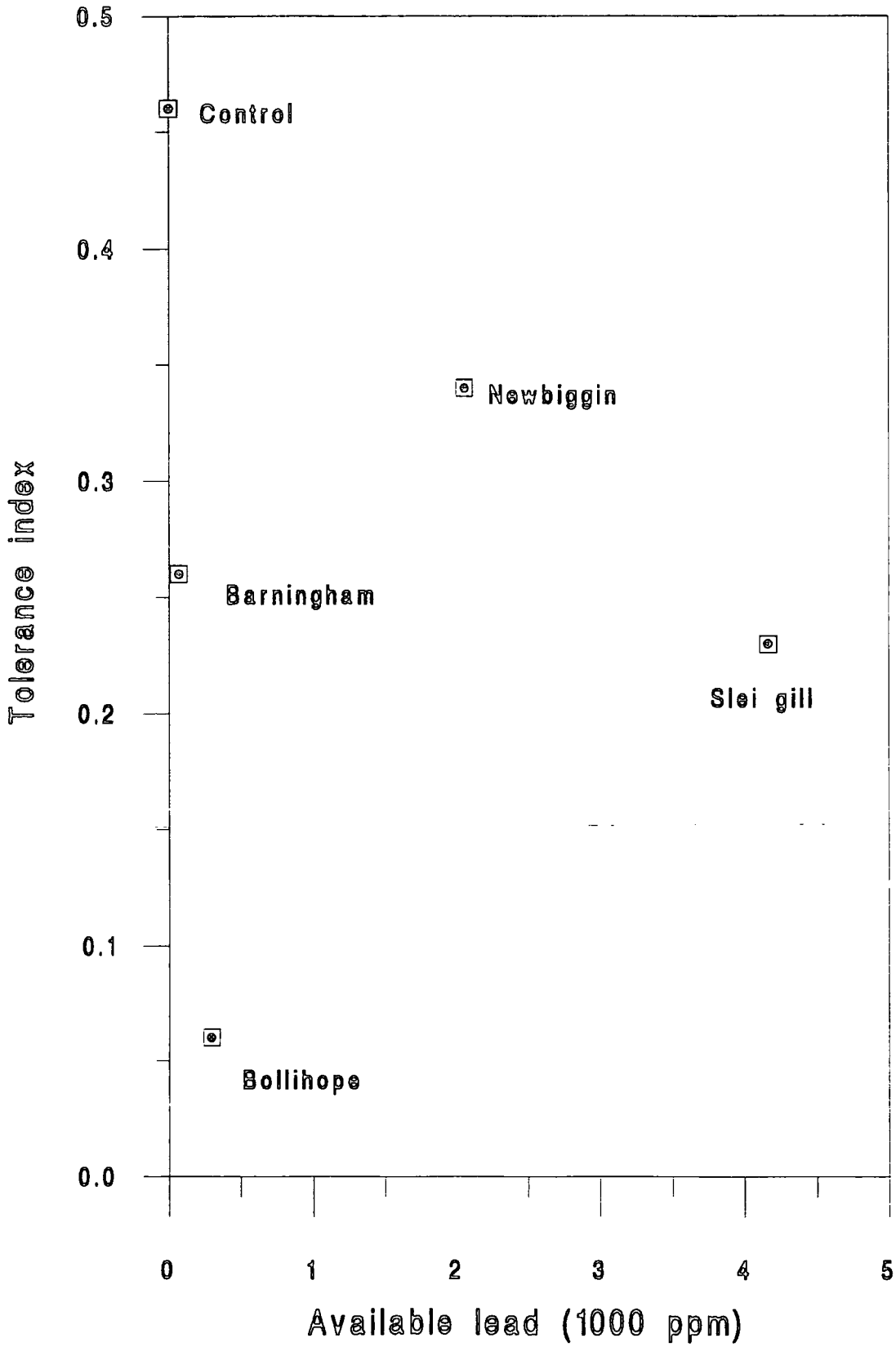


Fig 3. Relationship between available lead concentration at four sites and mean T.I. for plants from those sites.



Appearance of roots

Several changes were seen in the roots after treatment with lead. Features associated with a low T.I. were an increase in brittleness and rigidity so that lateral roots stood out at right angles from their origin, and a brown colouration on the root tips. A general browning of the roots was also associated with a low T.I., but a change from white to cream was not.

White root tips were usually associated with a high T.I. and indicated that the roots were still growing. In some cases new roots emerging from the rosette base had white tips, whereas the longer ones were brown. These plants appeared to have a low T.I. as the new short roots were not measured.

3.2.4. Discussion of results.

Barningham and Slei gill are both in the Northern Dales lead mining area. Barningham moor was chosen as a study site because it was at the same altitude as Slei gill, and because it was relatively distant from spoil heaps and mine sites. However the whole area has been a mosaic of lead contamination for centuries, (Raistrick 1972, Shayler *et al* 1978) and it is very probable that gene flow from tolerant *P.lanceolata* on contaminated sites has resulted in a higher than usual proportion of tolerant plants in unleaded sites. Bos *et al* (1986) measured gene flow in *P.lanceolata* at 0.2 - 1.4m per

generation. Over centuries, in an out-breeding perennial which flowers in its first year, such as *P.lanceolata* (Sagar and Harper 1958) this could result in gene flow over considerable distances and this would account for the unexpectedly high T.I.s found for some plants from Barningham.

The relatively low tolerance of some plants from the polluted soil in Slei gill is more difficult to explain. Possibly there are small pockets of unpolluted soil on the surface of contaminated heaps. Soil samples were taken from the rooting zone of the plants collected, but these were then mixed, and this might have obscured variations in lead content.

The plants used in this investigation had been transferred to garden soil 4-6 weeks previously. It is possible that *P.lanceolata* is capable of developing phenotypic tolerance (Baker *et al* 1986) and that 4-6 weeks in unpolluted soil has reduced this tolerance in some of the plants.

Approximately 20m from the contaminated Slei gill site there is a pasture with a plant community typical of uncontaminated soil which includes *P.lanceolata*. Baker (1987) suggests that intolerant ruderal and annual species may grow on polluted soil sporadically, with

reduced vigour. Morphological evidence suggests that *P.lanceolata* plants from Slei gill grow more slowly than those from Barningham. It is possible that the intolerant plants are the result of gene flow from nearby pasture, and that selection pressure is exerted by the lead at a later stage in their development, by slowing growth to such an extent that they reproduce less often than tolerant plants.

A further study of variation in the response of *P.lanceolata* to leaded soil could be undertaken by collecting a large sample of plants from one site, and propagating each plant to produce clones. In this way results of T.I. determinations could be replicated, and analysed for significant variation. It is possible to propagate *P.lanceolata* vegetatively from leaf cuttings (Wu and Antonovics 1975).

3.3.1. Results of proline estimations

Key to groups

Group A Slei gill plants in Slei gill soil
Group B Barningham plants in Slei gill soil
Group C Slei gill plants in garden soil
Group D Barningham plants in garden soil

Mean proline concentrations (μmoles g^{-1} fresh weight) in leaves from four groups of plants are shown in table 13.

Table 13. Mean proline concentrations.

Group	n	Conc.	SEM
A	4	5.38	0.62
B	4	11.90	1.39
C	4	11.99	0.43
D	5	13.19	1.40

Results of analysis of variance

	df	M	F	
Totals	16			
Groups	3	52.17	9.80	P= 0.001
Residual		38	5.32	

Results of t-tests

	B	C	D
A	P<0.05	P<0.0001	P<0.01
B	-	P>0.9	P>0.5
C		-	P>0.4

There is a significant difference between the proline concentrations in leaves from plants from Slei gill growing in Slei gill soil (group A) and the other three groups.

The calibration curve for proline determination is fig.11 in the appendix, and data for the determination is in table 3.3.1.i.

3.3.2. Discussion of results

Comparison of these results with those obtained by Hanley (1990) for *P.lanceolata* in polluted and unpolluted sites suggests that Groups B, C and D all have raised proline levels. The concentrations found in group A are similar to those recorded by Hanley.

As a planned part of this investigation, groups B and C were to be transplanted to the native soil of the other group. Because of the difficulty of obtaining soil from Barningham, garden soil was substituted. Although the difference in lead concentration was minimal, there were undoubtedly other differences in these soils, so that group D (from Barningham), which it was intended to leave in native soil, was also effectively transplanted to non-native soil. Transplantation took place eight weeks before the proline determinations were carried out. If raised proline levels are caused by stress, and if such transplantation is a cause of stress, this would explain the difference between group A and groups B, C and D, as group A was the only one not so transplanted.

Before the proline estimations, all the plants were in the greenhouse during a very hot weekend. Efforts were made to keep them well watered, but they may have been under stress. Stewart and Lee (1974) found

that there were higher levels of proline in plants transplanted to the laboratory and provided with a non-limiting nitrogen supply than in plants in the field in soil with very little nitrogen. Plants in group A have always grown in spoil heap soil, which is typically nutrient poor, and possibly this is the reason why their proline levels were lower than the other three groups, although all may have experienced stress.

3.4. Results of morphological studies

Plantago lanceolata plants from Barningham and Slei gill showed several morphological differences. Slei gill plants were smaller and the leaves were horizontal, flat and shiny. Few leaves had petioles. Barningham plants had nearly vertical leaves, many with petioles. The leaves tended to fold in half longitudinally.

Some of these features altered when the plants were transplanted into different soil. The Slei gill plants grew larger in garden soil, and some of the Barningham plants' leaves became horizontal when they grew in soil from Slei gill.

Key to plant groups

A Slei gill plants in Slei gill soil
B Barningham plants in Slei gill soil
C Slei gill plants in garden soil
D Barningham plants in garden soil

3.4.1. Results of measurements of leaf size

The mean length of the longest leaves from plants in groups A, B, C and D is shown in table 14.

Table 14. Mean lengths of leaves

Group	Mean leaf length (mm)	n	SEM
A	37.0	10	1.58
B	127.6	10	12.82
C	54.5	10	2.48
D	125.0	12	12.94

Results of analysis of variance

	df	M	F	
Total	41			
Groups	3	23138.60	23.34	P<0.001
Residual	38	991.26		

Results of t-tests

	B	C	D
A	P<0.001	P<0.001	P<0.001
B	-	P<0.001	P>0.8
C		-	P<0.001

The only pair not significantly different were groups B and D. These were the two groups of plants from Barningham. B had grown in Slei gill soil for four weeks, while D grew in garden soil, but their leaf lengths were still similar. This may have been because the leaves measured were already fully grown when the plants were transplanted, and could not change in size.

Groups A and D (plants from different sites) showed significant differences in leaf length, D (from Slei gill) having shorter leaves. Plants from Slei gill in garden soil (group C) had significantly longer leaves than Slei gill plants in native soil (group

A) after four weeks, indicating that their growth rate had increased.

To sum up, plants from Slei gill had shorter leaves than plants from Barningham, but their growth rate increased when they were transplanted into garden soil.

The mean width of the longest leaves from plants in groups A, B, C, and D is shown in table 15.

Table 15. Mean width of leaves

Group	Mean leaf width (mm)	n	SEM
A	6.5	10	0.54
B	12.0	10	0.37
C	11.7	10	0.7
D	14.0	12	0.90

Results of analysis of variance

	df	M	F	
Total	41			
Group	3	107.96	20.66	P<0.001
Residual	38	5.23		

Results of t-tests

	B	C	D
A	P<0.001	P<0.001	P<0.001
B	-	P>0.5	P>0.05
C		-	P>0.05

Group A leaves (from Slei gill plants in Slei gill soil) were significantly narrower than any other group. That is, plants from the two sites differed, and when Slei plants were transplanted to garden soil their leaves became wider. Slei plants grown in garden soil for four weeks (C) increased their leaf width to a point where they were no longer significantly different from Barningham plants in

garden soil (D). This contrasted with leaf length which was still significantly different in these two groups. This was because Slei gill plants had fewer leaves with petioles. *P.lanceolata* leaves develop petioles as they mature. There is not a sharp boundary between the petiole and the rest of the leaf, so that it was not possible to measure only the leaf. If a petiole was present it was included in the leaf length.

The mean ratio of leaf length to leaf width in groups A,B,C and D is shown in table 16.

Table 16. Mean ratio of leaf length to width.

Group	Ratio	n	SEM
A	5.929	10	0.383
B	10.526	10	0.884
C	4.805	10	0.313
D	9.142	12	0.942

Results of analysis of variance

	df	M	F	
Total	41			
Group	3	73.31	13.30	P<0.001
Residual	38	5.51		

Results of t-tests

	B	C	D
A	P<0.001	P<0.05	P<0.01
B	-	P<0.001	P>0.1

Groups B and D were not significantly different in any of these measurements. Leaf size and shape of plants from Barningham had not been affected by the difference in the soils they had been transplanted into. However four weeks in garden soil had decreased the ratio of length to width in leaves

from plants from Slei gill. (Groups A and C were significantly different).

Plants from Slei gill and plants from Barningham had a significantly different length/width ratio. Leaves from Barningham plants were longer in relation to their width, because more of them had developed petioles.

3.4.2. Results of measurements of specific leaf weight.

The mean specific leaf weight (SLW) of leaves from plants in groups A, B, C and D is shown in table 17.

Table 17. Mean specific leaf weights.

Group	SLW	n	SEM
A	0.2979	30	0.0065
B	0.2437	30	0.0063
C	0.2781	30	0.0069
D	0.2422	30	0.0076

Results of analysis of variance

	df	M	F	
Total	119			
Groups	3	0.02	15.92	P<0.001
Residual	116	0.00		

Results of t-tests

	B	C	D
A	P<0.001	P<0.05	P<0.001
B	-	P=0.001	P>0.5
C		-	P=0.001

SLW is a measure of leaf thickness and/or density.

Groups A and C were significantly different, that is, the SLW of Slei gill plants had decreased after four weeks in garden soil. Transplantation had had no effect on Barningham leaves (B and D are not significantly different). Group A is significantly different from the other groups, that is, Slei gill plants in native soil have a higher SLW than other groups.

3.4.3. Results of measurements of leaf number.

The mean number of leaves per plant in groups B, C and D is shown in table 18 . (Results were not obtained for group A)

Table 18. Mean number of leaves on each plant

Group	Number of leaves	n	SEM
B	7.10	10	0.43
C	8.30	10	0.62
D	7.33	12	0.51

Results of analysis of variance

	df	M	F	
Total	31			
Groups	3	4.10	1.39	P>0.2
Residual	29	2.95		

The number of leaves on each plant did not vary significantly in groups B C and D.

The mean proportions of leaves with petioles in groups B, C and D are shown in table 19 on the next page.

Table 19. Mean proportion of leaves with petioles.

Group	Proportion	n	SEM
B	0.6172	10	0.0496
C	0.1121	10	0.0434
D	0.6819	12	0.0275

Results of analysis of variance

	df	M	F	
Total	31			
Groups	2	1.02	60.02	P<0.001
Residual	29	0.02		

Results of t-tests

	B	C	D
B	-	P<0.001	P>0.1
C		-	P<0.001

Groups B and D were Barningham plants and were not significantly different. The proportion of leaves which had petioles was significantly less in group C (Slei plants) than in groups B or D.

Details of all these measurements can be found in the appendix, on tables 3.4.i, ii, and iii.

3.4.4. Discussion of results

This study was undertaken when it was realised that the morphological characteristics of some of the plants had changed four weeks after they were placed in non-native soil. For this reason data on these characteristics was not recorded when the plants were obtained, and the record of changes is incomplete as leaf number with and without petioles was not recorded for group A.

Comparisons between groups A and D showed that plants from Barningham had longer and wider leaves than plants from Slei gill, and that their SLW was lower. Comparisons between groups A and C showed that when Slei gill plants were transplanted into garden soil for four weeks their leaves became wider but not longer. The differences between the soils used to transplant Barningham plants had not affected their size and shape.

The number of leaves on the plants did not vary between the three groups for which it was recorded. When Slei gill and Barningham plants in garden soil were compared, Slei gill plants were found to have a lower proportion of leaves with petioles. In *P.lanceolata* only the older, outer leaves of the rosettes have petioles. This suggests that Slei plants grow at a slower rate than Barningham plants. They produce a similar number of leaves, but these

leaves take much longer to reach maturity. Flowering is initiated in *P.lanceolata* by long day length (Sagar and Harper 1964), but also depends on the size of the plant (Tonsor 1989). These results suggest that plants whose growth is slow for any reason will have a lower reproductive rate than fast growing ones. They also show that the growth rate of plants from Slei gill is much less than that of plants from Barningham.

The higher SLW found in Slei gill plants in native soil has several possible causes. SLW varies with the density and/or the thickness of the leaf, and changes in water content affect both of these. Density is also affected by the proportions of materials such as lignin and cutin in the leaf. Variation in these properties may affect the leaf's palatability.

3.5. Results of palatability studies

3.5.1. Pilot experiment to determine optimum conditions for palatability tests.

Table 20 shows how the weights (Wmg) and areas (Amm^2) of pieces of leaf changed over three days at different temperatures. Areas are shown in brackets.

Table 20

1. Changes at 15^{0}C.

Snail number	Days starved	W or A	First day	Second day	Third day
1	1	W	58	21	-3
		A	(26)	(22)	(0)
2	1	W	112	4	4
		A	(105)	(32)	(2)
3	2	W	13	-7	1
		A	(11)	(1)	(2)
4	2	W	15	22	32
		A	(69)	(67)	(136)
5	3	W	74	66	-3
		A	(300)	(300)	(0)
6	3	W	68	68	69
		A	(300)	(300)	(313)
Control leaf		W	23	36	-1
		A	(0)	(0)	(0)

The snails which were starved for three days either ate the whole leaf (300mm^2), or ate nothing. The snails starved for one or two days ate part of the leaf.

Changes at 20°C.

Snail number	Days starved	W or A	First day	Second day	Third day
7	1	W	60	7	-9
		A	(29)	(17)	(-8)
8	1	W	131	10	62
		A	(289)	(300)	(300)
9	2	W	69	50	51
		A	(212)	(228)	(0)
10	2	W	60	15	0
		A	(126)	(174)	(0)
11	3	W	0	24	53
		A	(0)	(300)	(222)
12	3	W	68	69	142
		A	(300)	(300)	(500)
Control leaf		W	-67	14	-10
		A	(0)	(0)	(0)

Snails which were starved for two or three days ate all or nearly all the leaf or nothing. One snail starved for one day ate part of the leaf, the other ate the whole leaf.

Changes at 25°C

Snail number	Days starved	W or A	First day	Second day	Third day
13	1	W	50	71	1
		A	(0)	(85)	(164)
14	1	W	98	5	59
		A	(0)	(0)	(0)
15	2	W	27	3	-14
		A	(75)	(80)	(75)
16	2	W	58	7	24
		A	(192)	(203)	(7)
17	3	W	75	97	210
		A	(300)	(300)	(600)
18	3	W	11	-12	4
		A	(26)	(44)	(47)
Contr. leaf		W	101	70	1

The control leaf area could not be measured as it had shrivelled. The leaves shrivelled rapidly at this temperature, and the snails ate erratically. This temperature appears to be unsuitable for this type of experiment.

Snail behaviour

The amounts eaten by snails varied widely under the same conditions. However they appeared to eat a measurable amount at 15^{0}C in one day if starved for one day previously. Higher temperatures and longer starvation times resulted in larger amounts being eaten, but this would have made it necessary to offer larger pieces of leaf in palatability tests. However there was a shortage of leaf material which precluded this. It would also have increased the probability that the leaves offered would be completely eaten, which would make it impossible to calculate a palatability index.

Leaf water content

There were two reasons for weight change in leaves placed with snails: the leaves were partly eaten, and their water content varied (shown by the control). Their areas changed when they were partly eaten, but not when their water content changed. For this reason,

the weights and areas in the table are not consistent with each other.

As a result of these observations, an investigation into the conditions needed to keep the leaves' water content constant was undertaken. The results of this are shown in table 21, which shows the volume of water added, and the mean % change in leaf weight. Full details of this experiment are in the appendix.

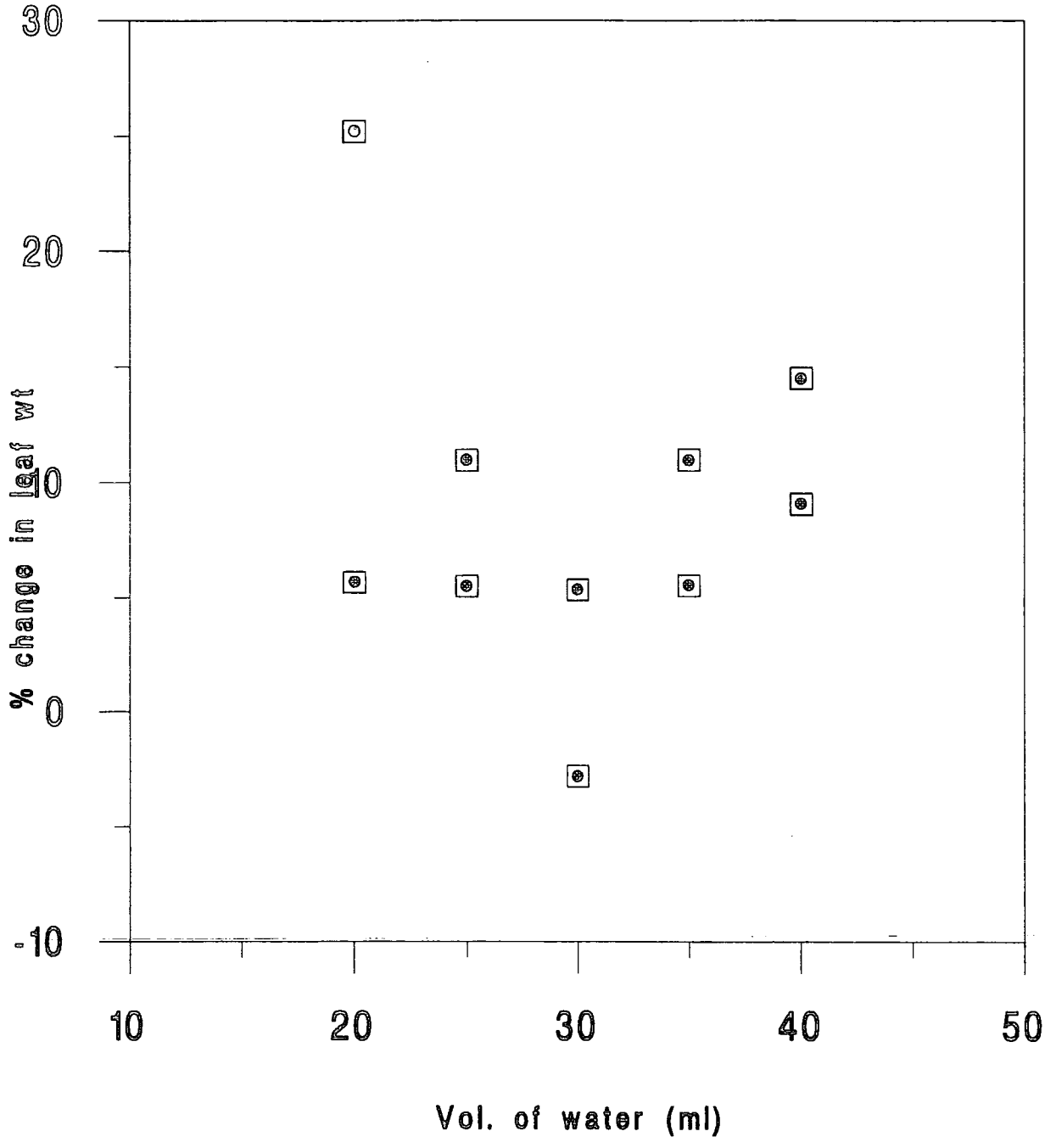
Table 21. % changes in leaf weight.

Box nos.	Vol of water(ml)	% change
1 and 2	20	+15.44
3 and 4	25	+8.23
5 and 6	30	+1.25
7 and 8	35	+8.25
9 and 10	40	+11.78

Fig 4 is a graph showing the % change in weight with different volumes of water. Although it was difficult explain these results, they showed that 30ml of water on the cotton wool in the boxes produced the least change in leaf weight over 24 hours.

These observations showed that it was possible to carry out the palatability tests at 15^{0}C, with a starvation time of one day, and a test duration of one day. These conditions were the most practicable, and were adopted. 30cm^{3} of water was placed in the containers in each box.

Fig 4. % change in leaf wt with vol. of water supplied



3.5.2. Behaviour of snails in palatability tests

The mean weight eaten by each snail in tests 1 to 6 is recorded in table 22.

Table 22. Mean weights eaten by snails

Snail number	Mean wt eaten(mg)	Snail number	Mean wt eaten(mg)
1	61.89	11	39.95
2	139.25	12	47.71
3	62.39	13	34.64
4	73.81	14	32.40
5	1.48	15	36.33
6	30.40	16	61.29
7	123.68	17	62.75
8	35.70	18	6.85
9	37.50	19	19.78
10	17.76	20	68.04

There were large variations between snails in the mean weight of leaf eaten. In particular, no.5 ate very little, and may have been senescent. No snail died during the tests.

Calculation of a palatability index removes variation caused by the differing weights eaten, and emphasises the snail's preference, and how marked it was.

$$\text{Palatability index (P.I.)} = \frac{\text{Wt of test leaf eaten}}{\text{Total wt of leaf eaten}}$$

A P.I. of 0.5 indicates that no preference was shown between the test material and the reference material. A P.I. of more than 0.5 indicates that the test material was preferred, the intensity of the preference increasing with P.I. A P.I. of less than 0.5 indicates that the test material was rejected, the intensity of this rejection increasing as P.I. decreases.

Detailed results for 8 palatability tests (weights and areas of leaf material offered and eaten, and P.I.s for individual snails) are in tables 3.5i, ii and iii in the appendix.

3.5.3. Control tests

Table 23. Mean P.I.s for two parts of the same leaf, D1 and D2

	Leaf tested	Ref leaf	n	Mean P.I.	SEM
Snails 1-10	D1	D2	8	0.5288	0.1469
	D2	D1	8	0.4713	0.1469
Snails 11-20	D1	D2	8	0.5275	0.1446
	D2	D1	8	0.4725	0.1446

The results were analysed using a paired t-test.

Snails 1-10 $P > 0.8$
Snails 11-20 $P > 0.8$

There was no significant difference in the P.I.s when snails were offered two similar parts of the same leaf. After a test a snail, and faeces, might be found in any part of the box, and leaves had sometimes been moved, suggesting that the snails moved about freely in the box, and came into contact with both leaves, and supporting the finding of a similar P.I. for similar leaves. This result for the control test thus validated significant choices made in later tests.

3.5.4. Palatability tests between leaves from four groups.

Key to groups:
Group A Slei gill plants in soil from Slei gill
Group B Barningham plants in Slei gill soil
Group C Slei gill plants in garden soil
Group D Barningham plants in garden soil

Table 24. Mean P.I.s for all palatability tests.

	Leaf tested	Ref. leaf	n	Mean P.I.	SEM
Test 1	A	C	7	0.2829	0.1258
	C	A	7	0.7171	0.1258
Test 2	D	B	10	0.3010	0.1251
	B	D	10	0.6990	0.1251
Test 3	C	D	8	0.2313	0.1299
	D	C	8	0.7688	0.1299
Test 4	A	D	10	0.1690	O.O904
	D	A	10	0.8310	0.0904
Test 5	C	B	8	0.4038	0.1352
	B	C	8	0.5962	0.1352
Test 6	A	B	7	0.1971	0.0648
	B	A	7	0.8029	0.0648

Analysis of results

The results in table 24 were regrouped by reference leaf, and ordered in these groups with the highest test leaf P.I. first. This is shown in table 25

Table 25. Results regrouped by reference leaf

	Leaf tested	n	Mean P.I.	SEM
1.Ref.leaf A				
	D	10	0.8310	0.0904
	B	7	0.8029	0.0648
	C	7	0.7171	0.1258
2.Ref.leaf B				
	C	8	0.4038	0.1352
	D	10	0.3010	0.1251
	A	7	0.1971	0.0648
3.Ref.leaf C				
	D	8	0.7688	0.1299
	B	8	0.5962	0.1352
	A	7	0.2829	0.1258
4.Ref.leaf D				
	B	10	0.6990	0.1251
	D	8	0.4725	0.1446
	C	8	0.2313	0.1299
	A	10	0.1690	O.O904

Part 1 of this table shows that the three other groups all had high P.I.s (considerably above 0.5) when tested against A. That is, all other groups were preferred to Slei plants in Slei soil.

Part 2 shows that the three other groups all had low P.I.s (under 0.5) when tested against B. That is, Barningham plants in Slei soil were preferred to all others.

Parts 3 and 4 show that when C or D were reference groups the scores were spread across 0.5, indicating that some groups were preferred to the reference group, and some rejected.

Fig. 5 shows results for all test groups and reference groups plotted on a bar chart. Figs 6-9 show the results for each reference group separately, with 95% confidence limits. They are all plotted to the same scale, to enable comparison.

Results in each part of this table were analysed, using ANOVA, and t-tests where necessary.

P.I.s for groups B, C, and D tested with reference to A

	df	M	F	
Total	23			
Groups	2	0.03	0.37	P > 0.5
Residual	21	0.08		

This test showed no significant differences between these 3 P.I.s, which were all more than 0.5. Plants in the other three groups were all preferred to Slei plants in Slei soil with a similar intensity.

P.I.s for groups A, C and D tested with reference to B

	df	M	F	
Total	24			
Groups	3	0.08	0.67	P >0.5
Residual	22	0.12		

This test showed no significant differences between these P.I.s, which were all less than 0.5. A similar intensity of preference for Barningham plants in Slei soil was shown in all tests.

Fig 5. Palatability indices for all groups

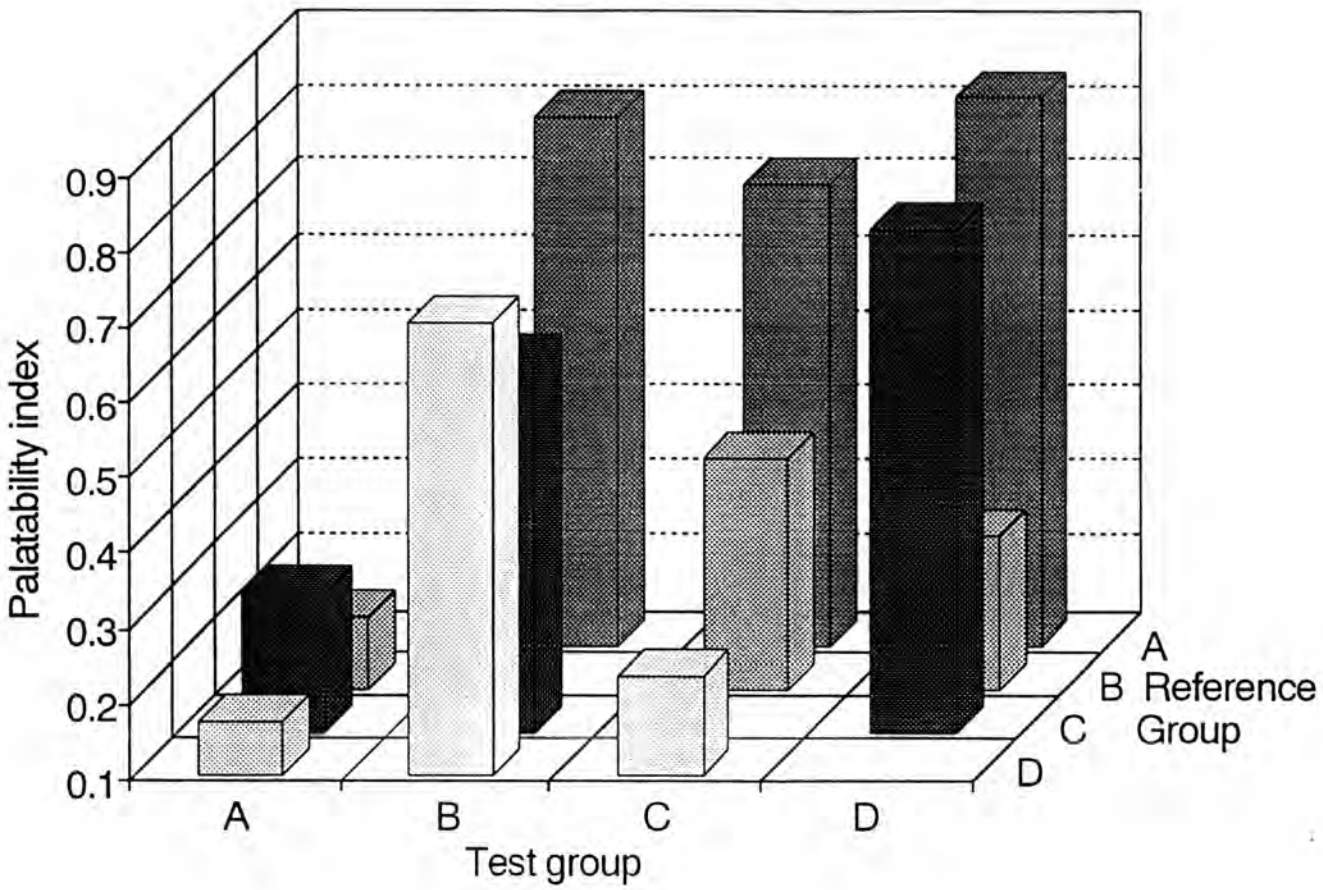


Fig 6. Palatability indices for groups B,C and D with reference to group A

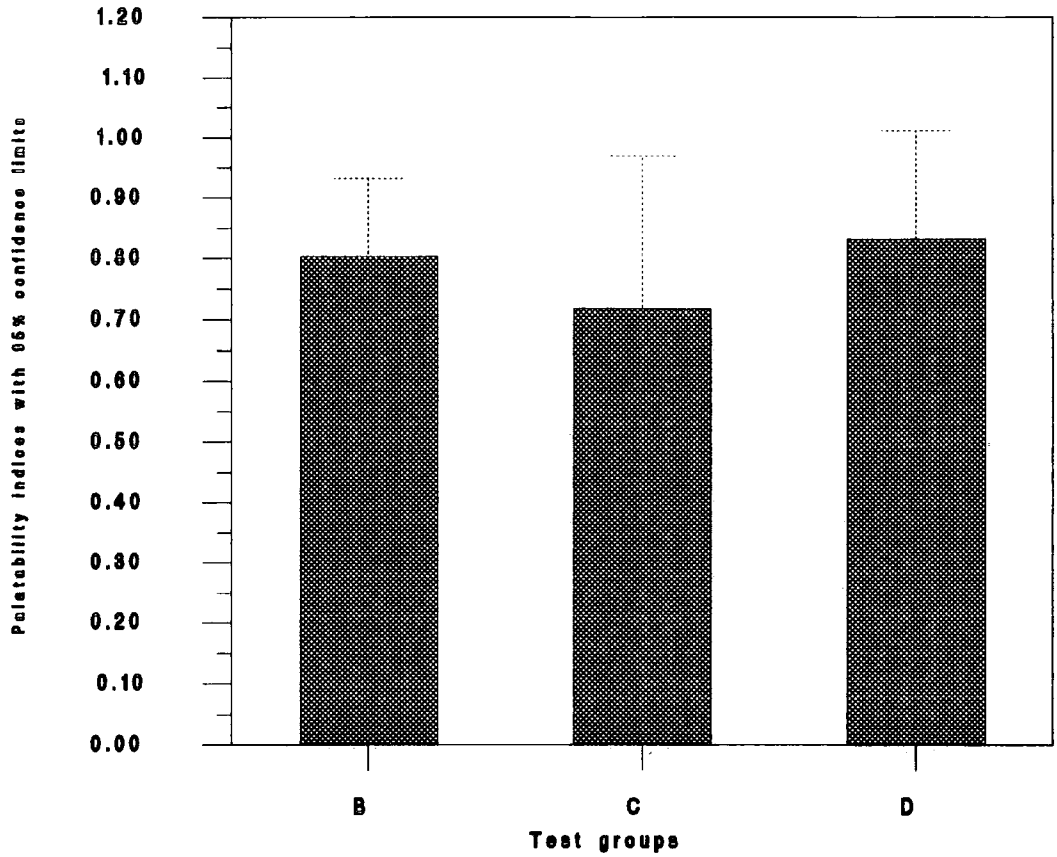


Fig 6. Palatability indices for groups A,C and D with reference to group B.

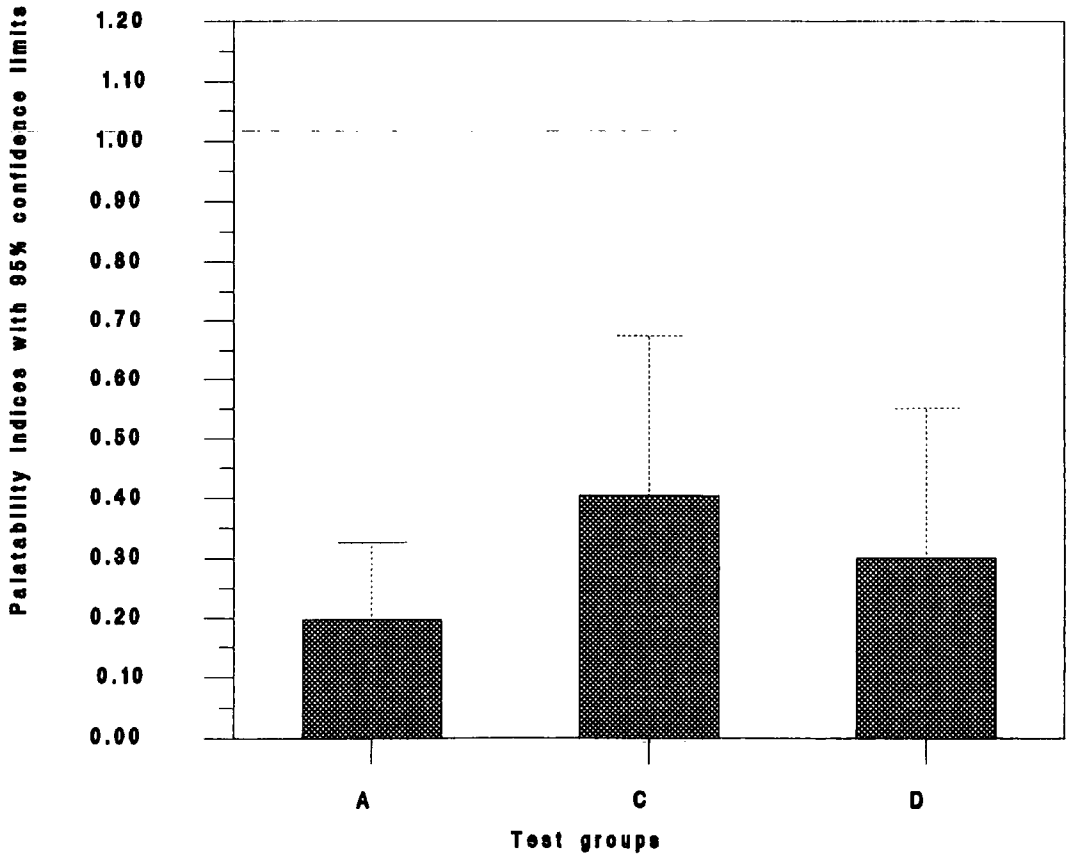


Fig 8. Palatability indices for groups A,B and D with reference to group C

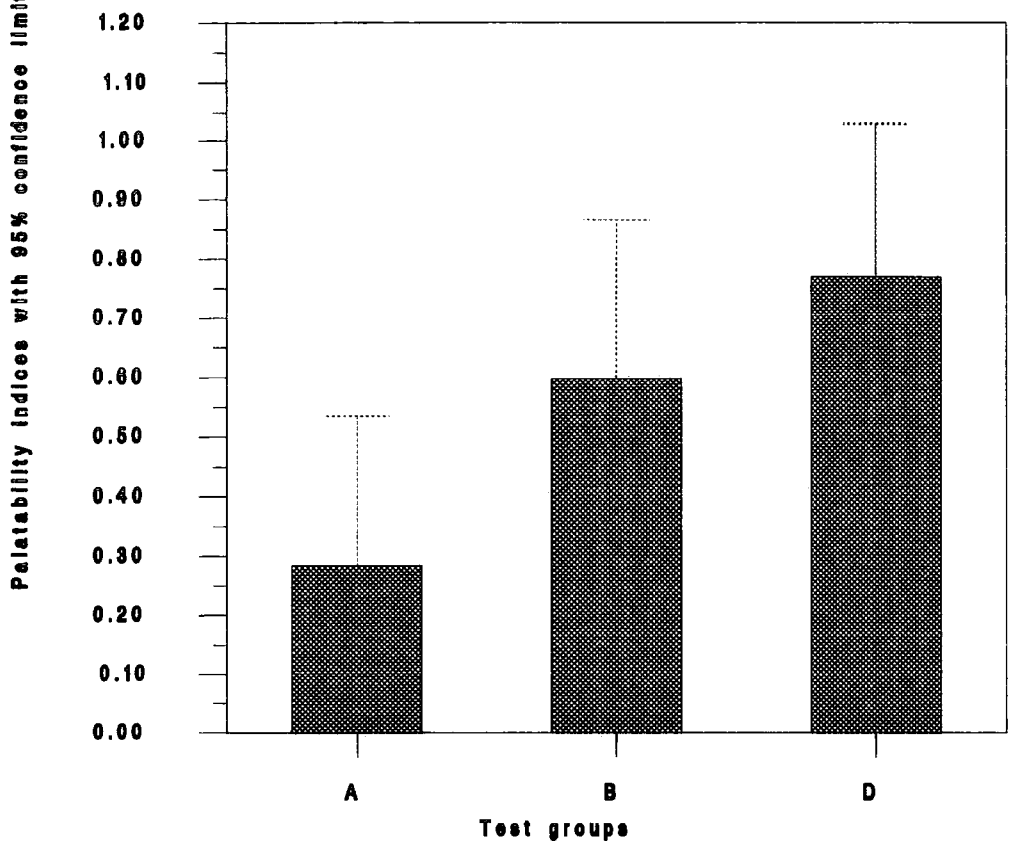
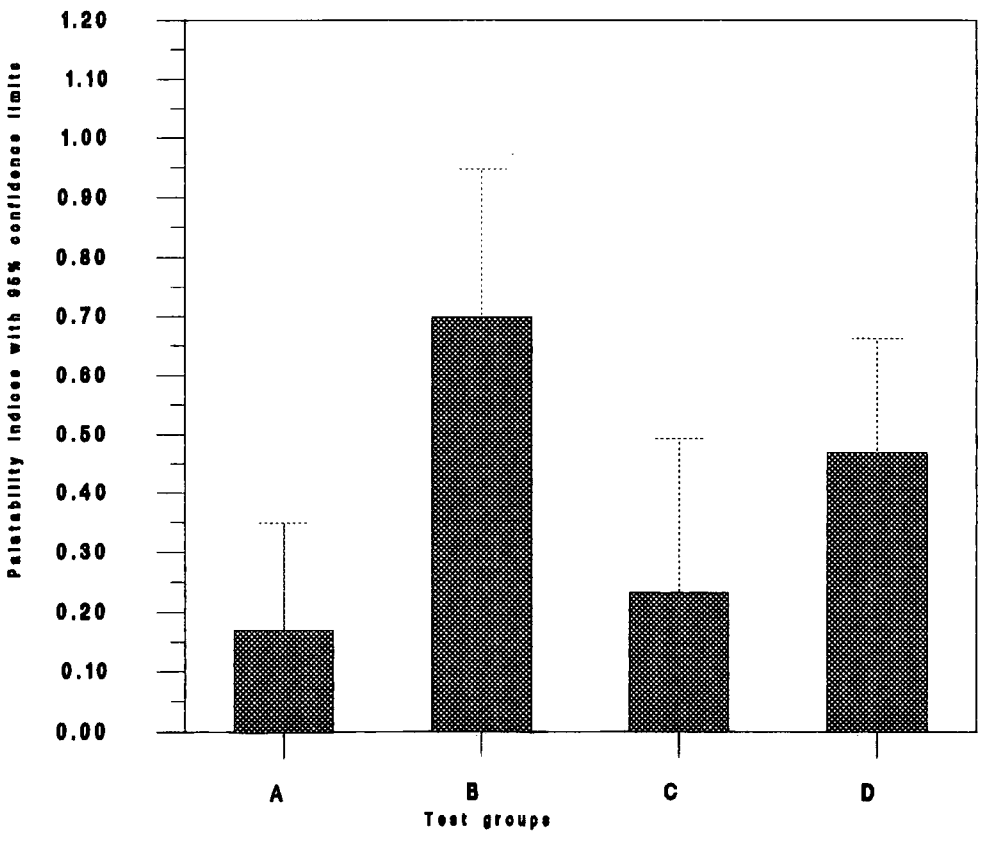


Fig 9. Palatability indices for groups A,B,C and D with reference to D



P.I.s for groups A, B and D with reference to C

	df	M	F	
total	22			
Groups	2	0.45	3.4	P = 0.05
Residual	20	0.13		

T-tests

	B	D
A	P>0.1	P<0.02
B	-	P>0.1

Group D had a significantly higher P.I than group A. That is, Barningham plants on garden soil were preferred, Slei plants on Slei soil were rejected, and the difference in degree of choice was significant when both were tested against Slei plants on garden soil.

As D was tested against D in the control test, it was possible to analyse the results for A, B, C and D with reference to D.

P.I.s for groups A, B, C and D tested with reference to D

	df	M	F	
Total	45			
Groups	3	0.58	4.07	P = 0.013
Residual	42	0.142		

T-tests

	B	C	D
A	P<0.005	P>0.5	P<0.05
B	-	P<0.05	P>0.1
C		P>0.1	

Group B had a significantly higher P.I. than Group A. That is, Barningham plants in Slei soil were preferred, and Slei plants in Slei soil were rejected, when both were tested against Barningham plants in garden soil.

Group D had a significantly higher P.I. than group A, showing that Barningham plants in garden soil(D) were preferred to Slei plants in Slei soil(A).

Group B had a significantly higher P.I. than group C. That is, when both groups of plants had been transplanted to non-native soil, Barningham plants (B) were still preferred.

3.5.4. Discussion of results.

Richardson and Whittaker (1982) found that varying the reference material varied the degree of discrimination between test species. In this investigation, all groups were treated as reference material in turn, and the results for group D provided the best discrimination between groups. Three significant differences have been established. They are that groups D and B were preferred to group A, and group B was preferred to group C. This suggests a preference ranking of B C D A or B D C A. This investigation was planned on the assumption that garden soil and Barningham soil were similar, and that plants transplanted between them would be unaffected. The results of proline determinations suggest that this may not be so, and make interpretation of these results more diffcult.

4. Discussion

The two sites from which plants were collected were selected because they differed in soil lead concentration. It was expected that the high lead concentration in Slei gill spoil heaps would have resulted in the evolution of a lead tolerant ecotype of *P.lanceolata*, and that plants from Barningham, where the lead level was low, would be less tolerant of lead (Baker 1988). This relationship was not demonstrated in the present study, although study of two intermediate sites provided evidence of a trend in this direction. There appears to be great variability in tolerance at both the sites, and the sample size was too small to resolve this. Thus there is no evidence for or against a relationship between lead tolerance and palatability to *H.aspersa* or between lead tolerance and raised proline levels in *P.lanceolata*.

The study was planned as an investigation into the differences between plants from the two sites when each were grown in their own and in the others' soil. Thus there were four groups of plants to be compared, with two variables, site of origin and source of present soil. Sufficient soil could not be obtained from one of the sites, and as it was the lead concentration in the soils that was under investigation for ite effect on the plants, soil

from elsewhere with a broadly similar lead content was substituted. As a result of this, another variable was unintentionally introduced, in that plants either continued to grow in their native soil, or were transplanted out of it. In order to clarify the different treatments, each possible pair of groups was considered, and comparisons of treatments were made within each pair. The table below summarises differences between the members of a pair in the three treatments: transplant history, origin of plants, and the soil in which they were planted.

Key to plant groups

A Slei gill plants growing in Slei gill soil.
B Barningham plants growing in Slei gill soil
C Slei gill plants growing in garden soil.
D Barningham plants growing in garden soil.

Table 26.
Differences in treatments between members of a pair

Pair	Transplanted	Slei origin	Slei soil
A+C	C	=	A
B+D	=	=	B
C+D	=	C	=
A+B	B	A	=
A+D	D	A	A
B+C	=	C	=

= indicates that both groups were similar.

Several characteristics of the leaves of plants in these groups were studied: their palatability to *Helix aspersa*, their proline concentration, and their size, shape and specific leaf weight (SLW). The growth rate of the plants was estimated using

the proportion of their leaves which had petioles. The lead content of the leaves was measured, but due to the shortage of plant material, only one sample from each group was analysed, so that the results were inadequate, and could not be used.

Each possible pair of groups was considered for differences in these characteristics between its members. The table below summarises the significant differences between the members of each pair.

Table 27
Differences between members of pairs, with regard to the characteristics shown.

Group	Prol	Pal	L.l	L.w	G.r	SLW
A+C	C	=	C	C	?	A
B+D	=	=	=	=	=	=
C+D	=	=	D	D	D	C
A+B	B	B	B	B	?	A
A+D	D	D	D	D	?	A
B+C	=	B	B	=	B	C

Key
= no significant difference detected.
? insufficient data.
Prol proline
Pal palatability
L.l leaf length
L.w leaf width
G.r growth rate
SLW specific leaf weight

The capital letters in the table denote the group with the higher result in each case.

Comparisons were made within pairs, using the above table, and relationships were particularly sought between palatability and other characteristics. Three such relationships were identified.

Firstly, it can be seen that proline concentration and palatability are directly related in two pairs (A+B, A+D). In both cases the group with the higher proline content is also the more palatable one. Secondly, palatability and SLW have an inverse relationship in three pairs (B+C, A+D, and A+B). In these pairs, leaves with a higher SLW are less palatable to *Helix aspersa*. Thirdly, leaf length and width are directly related to palatability in two pairs (A+B and B+C), in which larger leaves are more palatable than smaller ones.

It is therefore possible that proline concentration, SLW, and leaf size all affect palatability, or that one or two of them do, or that all three and palatability are affected by some other factor. The snails had been offered pieces of leaf in the palatability tests, and it was thought that this made it unlikely that the size and shape of whole leaves could have affected their choices, and therefore leaf size was not considered further as a cause of palatability,.

Comparisons were made between tables 26 and 27, with particular reference to proline concentration and SLW. These showed a relationship between proline concentration and transplantation in all pairs. In each pair, if only one had been transplanted, it had the higher proline level. The tables also showed a link between high SLW and Slei origin in three

pairs. Plants from Slei gill had leaves with a high SLW.

Thus it seems probable that a plant's origin, and its transplantation history, affect its SLW and its proline concentration respectively, and that SLW and proline content may affect palatability.

The results show that transplantation eight weeks previously is a possible cause of raised proline levels. Proline accumulation is an indicator of stress, and transplantation is probably stressful.

If the two groups of plant from the unpolluted site are compared, it can be seen that their proline levels were equally high. The group transplanted to soil with a high lead content (B) did not have a higher proline level than the group moved to soil with a low lead content (D). Thus lead concentration in the soil did not appear to affect proline concentration in these plants.

Further evidence of the effects of lead on proline concentration is provided by the significantly lower proline levels in group A than the other three groups. This difference may have been because group A plants were not transplanted, but the low level in group A suggests that they were not stressed by the polluted soil. Saradhi (1991) found that seedlings (not *P.lanceolata*) grown in leaded media had raised proline levels, in contrast to the plants in group A. *P.lanceolata* plants from unpolluted sites were

watered with lead solution by Hanley (1990), and were found to have raised proline levels, in contrast to group B. The variability of plants from both these sites with regard to lead tolerance has been noted, and it is possible that some or all of the plants from either site were lead tolerant.

Proline may have a function in the plant's response to stress, or its accumulation may be a result of stress. If the latter is true, it would be expected that a tolerant plant would produce less proline under stress than an intolerant one. Pearson and Stewart (1991) compared proline levels in the grain of three varieties of barley under different degrees of water stress, and found that the most drought tolerant variety had the lowest proline levels. On the other hand, Stewart and Lee compared saline tolerant and intolerant ecotypes of *Armeria maritima*, and found that the tolerant populations had the higher proline levels. The results in this study indicate that tolerant plants may have had lower proline levels.

This suggests that the Slei gill plants were acclimatised in some way to lead polluted soil, although this was not always expressed in root elongation in tolerance tests. This acclimatisation was not complete, as the results of leaf measurements showed that plants from polluted soil were smaller than those from unpolluted soil, and grew more slowly. It must be remembered that plants

on spoil heaps suffer from, and must be tolerant to, a range of stress producing factors. Their slow growth could have been the result of low nutrient levels in the soil (Jowett 1959), or of a root system which had been prevented from developing adequately by high concentrations of lead, and absorbed nutrients poorly.

If *Helix aspersa* has evolved the ability to detect differences in *P.lanceolata* leaves, it is because it benefits by eating them selectively. There may be advantages in eating leaves with a high proline concentration, or with a particular SLW.

Hopkins (1989, quoting Walther *et al* 1984) reported an increased rate of reproduction in aphids living on beans following application of low concentrations of lead to the plants. He interpreted this as a response to increased nitrogen levels in the phloem sap following metal induced stress. An increase in proline concentration of the magnitude observed here will, if it is not accompanied by a decrease in the precursors of proline, increase the nitrogen content of *P.lanceolata* leaves, and thus improve their quality as food.

The benefit a snail obtains from a leaf may also be related to the leaf's SLW. SLW is a measure of the leaf's density, and/or thickness. The density of a leaf depends on the relative proportions of its

contents, so that variations in water content will affect it, as will the proportion of such substances as lignin and cutin. These factors will also affect the value of the leaf as food. Leaf thickness, the other characteristic affecting SLW, might affect the speed or ease with which a leaf could be eaten.
Since *Helix aspersa* would encounter leaves with raised proline levels in a wide range of food plants, whereas the effects of SLW changes are more variable, variation in proline levels seems the most probable cause of the evolution of selectivity.

The relationship between raised proline levels and palatability in *P.lanceolata* raises ecological questions. It suggests that plants under stress are at greater risk from predation by herbivores such as *H.aspersa* than plants which are not stressed. A future study of a range of plant species under stress, and a range of herbivores which feed on them, might investigate how widespread the relationship between proline concentration and palatability was, and the extent of its effect on the plants.
In conclusion, this study has shown that growth in lead polluted soil does not always cause high proline levels in *Plantago lanceolata*. However when such levels do occur in *Plantago lanceolata* leaves their palatability to *Helix aspersa* may be increased.

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APPENDIX

Contains tables

Table 3.1 i.

Determination of lead concentrations of soil samples.

HCl extraction.

Sample	Wt(g)	abs.	ppm	dil.	soil.ppm
Slei 1	5.01	36.00	1.99	1,000	5,953.33
Slei 2	5.34	34.00	1.91	1,000	5,362.16
Boll 1	4.75	32.00	1.83	100	578.07
Boll 2	4.89	22.00	1.42	100	436.02
Newb 1	5.64	34.00	1.91	500	2,537.64
Newb 2	5.26	31.00	1.79	500	2,550.40
Barn 1	5.29	84.00	3.94	10	111.83
Barn 1	5.53	83.00	3.90	10	105.83
Gard 1	5.47	40.00	2.15	50	295.28
Gard 2	5.19	42.00	2.24	50	323.37

EDTA extraction

Sample	Wt(g)	abs.	ppm	dil.	soil.ppm
Slei 1	4.60	56.00	2.81	500	4,574.36
Slei 2	5.20	51.00	2.60	500	3,755.77
Boll 1	5.21	38.00	2.07	50	298.36
Boll 2	4.89	33.00	1.87	50	286.87
Newb 1	5.67	60.00	2.97	250	1,961.61
Newb 2	5.02	58.00	2.89	250	2,154.59
Barn 1	5.59	96.00	4.43	5	59.49
Barn 2	5.15	101.00	4.64	5	67.51
Gard 1	5.22	46.00	2.40	25	172.13
Gard 2	5.27	44.00	2.32	25	164.78

Calibration data for lead concentration

Conc of lead (ppm)	Absorbance
1	19
2	29
3	58
4	88
5	110

Fig.10. Calibration graph for lead determination

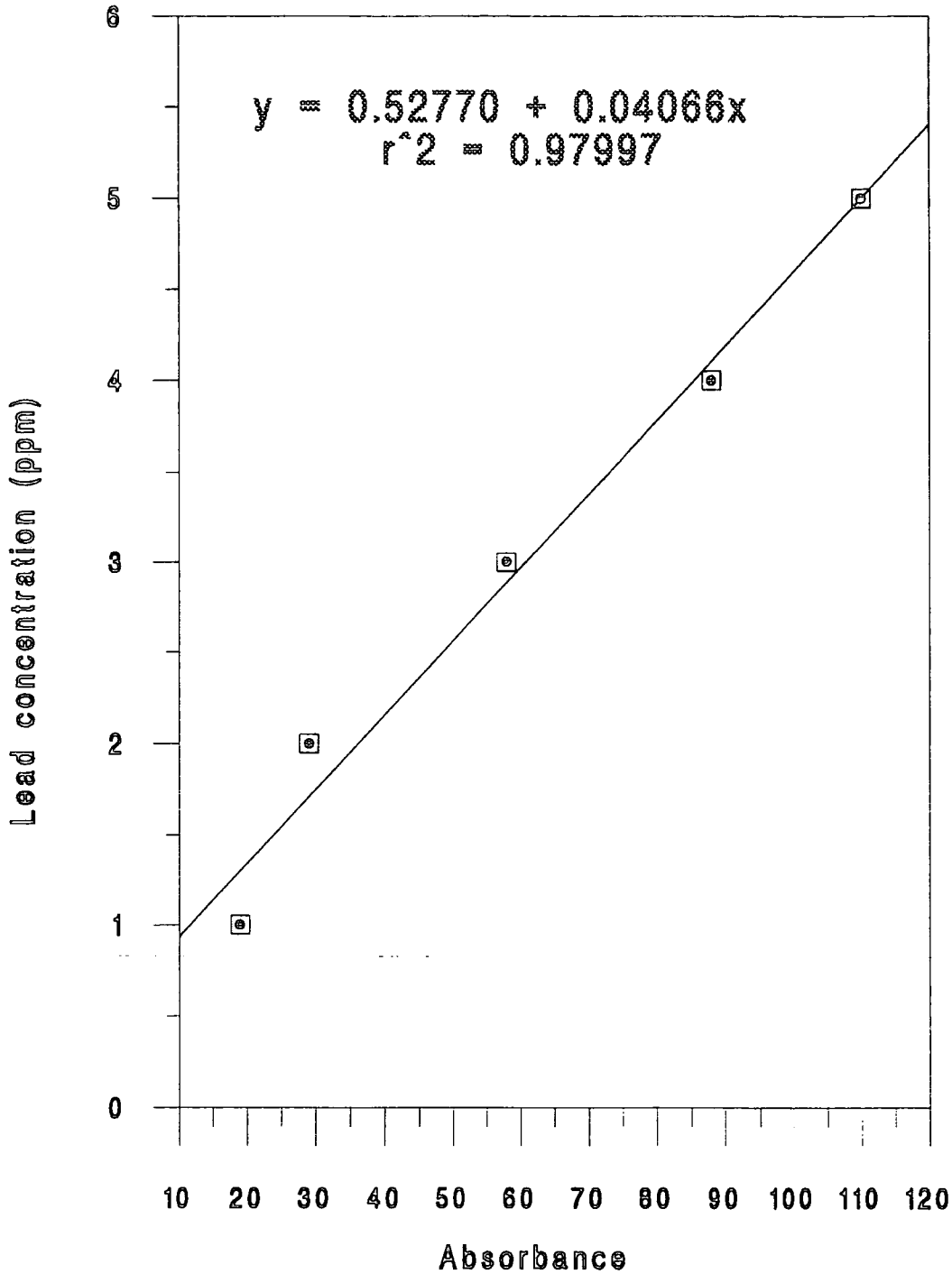

Table 3.2.1.i. Five Moulds plants

Lengths of longest roots measured over 12 days.

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Mean
1	50	53	55	43	31	46.4
2	50	52	55	44	33	46.8
3						
4	56	48	57	53	33	49.0
5						
6						
7	78	76	70	68	59	70.2
8	85	81	75	72	71	76.8
*9	94	88	80	78	76	83.2
10	97	87	80	78	79	84.2
11	98	90	84	79	77	85.6
12	98	89	83	77	76	84.6
13						
14	96	86	82	81	74	83.8

*25ppm lead added

T.I. of five Moulds plants

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Mean
TI	0.17	0.05	0.20	-0.07	0.00	0.07

Table 3.2.2.i. Determination of Optimum lead concentration

Bollihope plants

Mean lengths of three longest roots on 16 days

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
1		43.33	68.33		
2		45.67	73.00		69.67
3					
4	48.00	48.67	83.00		74.67
5	52.00	49.33	100.67		82.00
6	61.00	48.33	108.67	47.00	83.33
7	68.33	46.67 new	118.67	52.00	83.00
8	71.67	44.33	125.00	57.00	86.33
9					
10					
11	91.67	65.33	136.67	85.67	89.00
*12	96.33	78.33	143.67	95.33	90.00
13	99.33	84.67	145.67	100.67	91.00
14	100.33	94.33	143.00	102.33	88.00
15	101.67	95.67	144.33	107.33	89.33
16	101.33	93.00		107.33	88.00

*2.5ppm lead

Five Bollihope plants

Mean lengths of three longest roots on 12 days

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
1	53.33	38.67	69.67		54.00
2	54.67	39.00	70.00	63.00	55.00
3	49.33	40.00	68.67	64.00	55.33
4	57.33	38.33	70.67	62.33	52.67
5					
6	62.00	39.67	85.67	64.33	57.00
**7	66.67	43.00	90.67	67.33	62.33
8	68.67	43.33	91.33	67.67	65.33
9		45.67	97.33	66.67	63.33
10		44.33	95.67	66.33	63.33
11					
12		45.33	94.33		62.33

**5ppm lead

Table 3.2.2.i cont.

Barningham plants

Mean length of three longest roots on 21 days

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
1	33.33	29.00	46.00	38.00	48.00
2					
3					
4	51.67	42.67	72.00	50.33	50.00
5	55.00	50.00	77.33	54.33	48.67
6	60.33	56.00	79.33	59.33	53.67
7	66.00	63.00	85.67	66.67	59.67
*8	72.33	70.33	91.33	73.33	59.67
			new		
9	82.67	77.33	90.67	80.33	68.33
10	85.33	82.67	98.33	83.00	70.00
			new		
11	86.67	85.00	60.33	85.00	73.67
**12	91.33	88.67	61.33	87.33	76.67
13	92.33	95.33	62.33	87.67	81.33
14	100.67	97.67	64.00	85.67	83.67
15	104.00	98.33	59.67	86.33	84.33
16					
17	105.67	105.00	60.00	91.33	82.67
****18	106.33	106.67	62.00	90.67	84.67
19	105.00	106.67	62.33	90.67	84.33
20	109.33	103.33	62.33	91.33	84.00
21	108.67	104.67	61.33	93.33	84.67

*2.5ppm lead **5ppm lead ****10ppm lead.

Table 3.2.2.i cont.

Slei Gill plants

Mean lengths of three longest roots on 20 days

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
1	48.00	53.67		58.00	59.00
2	48.67	55.00		56.00	58.00
3	50.33	59.00	55.33	56.67	57.00
4	55.00	63.33	52.67	52.33	56.67
5	63.33	70.67	54.33	52.67	59.67
6	66.33	77.33	56.67	58.00	63.33
7	71.33	84.67	62.00	60.33	62.67
**8	75.33	87.33	70.33	67.00	61.33
9	78.67	94.67	76.33	65.00	61.33
9			new73.67		
10	77.67	94.00	73.67	65.67	60.67
11	78.00	95.33	74.00	66.67	59.33
12					
13	77.67	95.33	74.67	66.67	60.33
14	79.00	94.67	77.33	68.00	62.33
15	77.67	93.67	76.33	68.33	61.67
16	78.00	91.00	77.00	67.33	62.67
****17	76.33	94.67	73.33	67.67	62.67
18					
19	76.67	97.00	77.33	69.67	62.33

**5ppm lead ****10ppm lead

Table 3.2.2.i. cont.

Newbiggin plants

Mean lengths of three longest roots on 17 days

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant5
1		39.33	32.33	47.33	
2	53.00	45.33	42.67	60.33	31.00
3	61.33	57.67	52.67	72.33	38.00
4					
5					
6	100.67	94.00	85.33	93.33	86.67
*7	112.33	101.00	96.67	101.00	95.67
8	120.33	107.33	102.67	107.33	105.00
9	123.67	109.33	104.67	115.00	111.67
**10	125.00	110.67	107.67	121.67	117.33
11	124.67	113.00	107.67	123.67	122.33
12	126.33	117.00	109.67	125.00	128.67
12	new81.67			new64.00	
13	81.33	117.00	108.67	66.33	128.33
****14	81.33	121.00	113.00	66.67	133.67
15	79.33	122.00	113.67	66.33	133.33
16	79.67	124.00	114.00	67.00	134.33
16					new106.00
17	81.67	119.67	113.67	67.00	110.00

*2.5ppm lead **5.0ppm lead ****10.0ppm lead

Barningham plants (Control)

Mean lengths of 2 or 3 longest roots on 11 days

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
1	63.00	61.00	66.00	63.00	60.67
2	62.33	60.33	61.67	63.33	60.00
3	65.33	62.00	65.67	67.33	55.33
4					
5	63.67	64.33	70.00	67.67	60.33
6	66.00	67.67	70.33	67.00	58.33
7	68.67	75.00	78.00	69.50	59.00
8	69.67	80.00	80.50	70.50	60.33
9	69.00	83.67	76.00	68.50	62.33
10					
11	78.67	91.67	78.50	72.00	62.67

Plants 1 - 3 were aerated daily for 15 minutes.
Plants 4 - 5 were not aerated.
After day 5 root lengths of plant 4 are the mean of two measurements.
After day 7 root lengths of plant 3 are the mean of two measurements.
All other root lengths are the mean of three measurements.

Table 3.2.2.i cont

Provisional T.I.s for plants from four sites for three concentrations of lead

Bollihope plants

	Plant 1	Plant2	Plant3	Plant4	Plant 5	Mean
*T.I.	0.30	0.64	0.05	0.42	-0.24	0.23

Bollihope plants

	Plant 2	Plant 3	Plant 4	Plant 5	Mean
**T.I.	0.29	0.25	-0.20	0.10	0.16

Barningham plants

	Plant 1	Plant 2	Plant 4	Plant 5	Mean
*T.I.	0.83	0.72	0.61	1.27	0.81
**T.I.	0.73	0.48	-0.05	0.70	0.43
****T.I.	0.13	-0.10	0.14	0.00	0.04

Slei gill plants

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Mean
**T.I.	0.19	0.40	0.37	-0.09	-0.40	0.21
****T.I.	0.06	0.13	-0.03	0.12	-0.20	0.06

Newbiggin plants

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Mean
*T.I.	0.33	0.31	0.33	0.95	0.52	0.46
**T.I.	0.03	0.20	0.03	0.26	0.27	0.15
****T.I.	0.01	-0.04	0.02	0.02	0.11	0.03

* 2.5ppm lead **5ppm lead ****10ppm lead

Table 3.2.3.i. Determination of Tolerance Indices.
Root growth in plants from four sites and control.

2.5ppm lead added day 5

Day	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Mean
Slei plants.						
1	66.67	49.00	70.33	60.67	22.67	53.87
2	68.33	49.83	75.00	67.00	25.17	57.07
3	70.00	50.67	79.67	73.33	27.67	60.27
4	70.83	50.50	83.67	79.33	32.50	63.37
5	71.67	50.33	87.67	85.33	37.33	66.47
6	71.00	48.67	91.00	87.00	38.00	67.13
7	69.67	48.67	93.00	87.33	39.67	67.67
8	69.33	50.00	96.67	88.67	40.67	69.07
9	69.33	51.00	99.67	91.67	42.00	70.73
Newbiggin plants.						
1	86.67	62.33	24.00	61.33	37.67	54.40
2	89.00	63.83	25.50	62.67	40.83	56.37
3	91.33	65.33	27.00	64.00	44.00	58.33
4	96.50	65.83	29.00	66.33	46.50	60.83
5	101.67	66.33	31.00	68.67	49.00	63.33
6	107.67	66.33	32.50	71.67	49.00	65.43
7	111.33	67.67	33.50	72.33	49.00	66.77
8	111.67	64.67	32.50	72.33	49.33	66.10
9	113.33	65.00	33.50	72.33	51.00	67.03
Bollihope plants						
1		72.00	66.00	25.33		50.21
2		76.50	69.17	32.67		54.27
3		81.00	72.33	40.00		58.33
4		87.50	73.83	43.17		61.13
5		94.00	75.33	46.33		63.92
6		94.50	74.33	47.00		64.08
7		92.50	76.00	47.33		64.33
8		92.00	75.67	48.00		64.79
9		95.00	76.00	48.67		66.67
Barningham plants						
1	43.33	85.33	35.67	31.33	41.67	47.47
2	48.50	88.67	40.83	33.00	44.00	51.00
3	53.67	92.00	46.00	34.67	46.33	54.53
4	60.50	96.33	50.50	38.33	48.83	58.90
5	67.33	100.67	55.00	42.00	51.33	63.27
6	71.00	101.33	55.67	45.00	54.33	65.47
7	75.33	101.00	55.33	41.67	55.00	65.67
8	76.33	102.33	58.00	41.67	54.67	66.60
9	76.33	102.33	58.33	42.67	54.67	66.87

Control. Barningham plants. No lead added.

Day	Plant1	Plant2	mean
1	67.33	55.33	61.33
2	67.50	57.83	62.66
3	67.67	60.33	64.00
4	67.00	58.33	62.67
5	69.50	59.00	64.25
6	70.50	60.33	65.42
7	68.50	62.33	65.42
8	70.25	62.50	66.38
9	72.00	62.67	67.33

Table 3.3.1.i. Determination of proline concentrations

Gp	Samp. no.	Mean abs	Samp. conc $\mu g\ cm^{-3}$	Samp. wt(g) g	Acid vol(ml) ml	Leaf conc $\mu moles\ g^{-1}$
A	14	0.0083	9.51	0.36	15	6.86
	15	0.0040	5.77	0.36	15	4.16
	16	0.0047	6.35	0.48	20	4.58
	17	0.0073	8.65	0.38	15	5.91
B	10	0.0140	14.37	0.51	25	12.19
	11	0.0150	15.22	0.35	15	11.29
	12	0.0210	20.30	0.57	25	15.42
	13	0.0117	12.37	0.37	15	8.69
C	5	0.0183	18.05	0.39	15	12.02
	6	0.0137	14.08	0.23	12	12.72
	7	0.0137	14.08	0.34	15	10.76
	8	0.0140	14.37	0.3	15	12.44
D	9	0.0113	12.09	0.53	25	9.87
	2	0.0170	16.92	0.57	20	10.28
	3	0.0247	23.39	0.61	20	13.28
	4	0.0263	24.78	0.55	20	15.60

Calibration data for proline concentration

Conc. of proline ($\mu g\ cm^{-3}$)	Absorbance
5	0.002
10	0.005
25	0.024
50	0.062
100	0.140
200	0.271
250	0.437

Fig 11. Calibration graph for proline determination

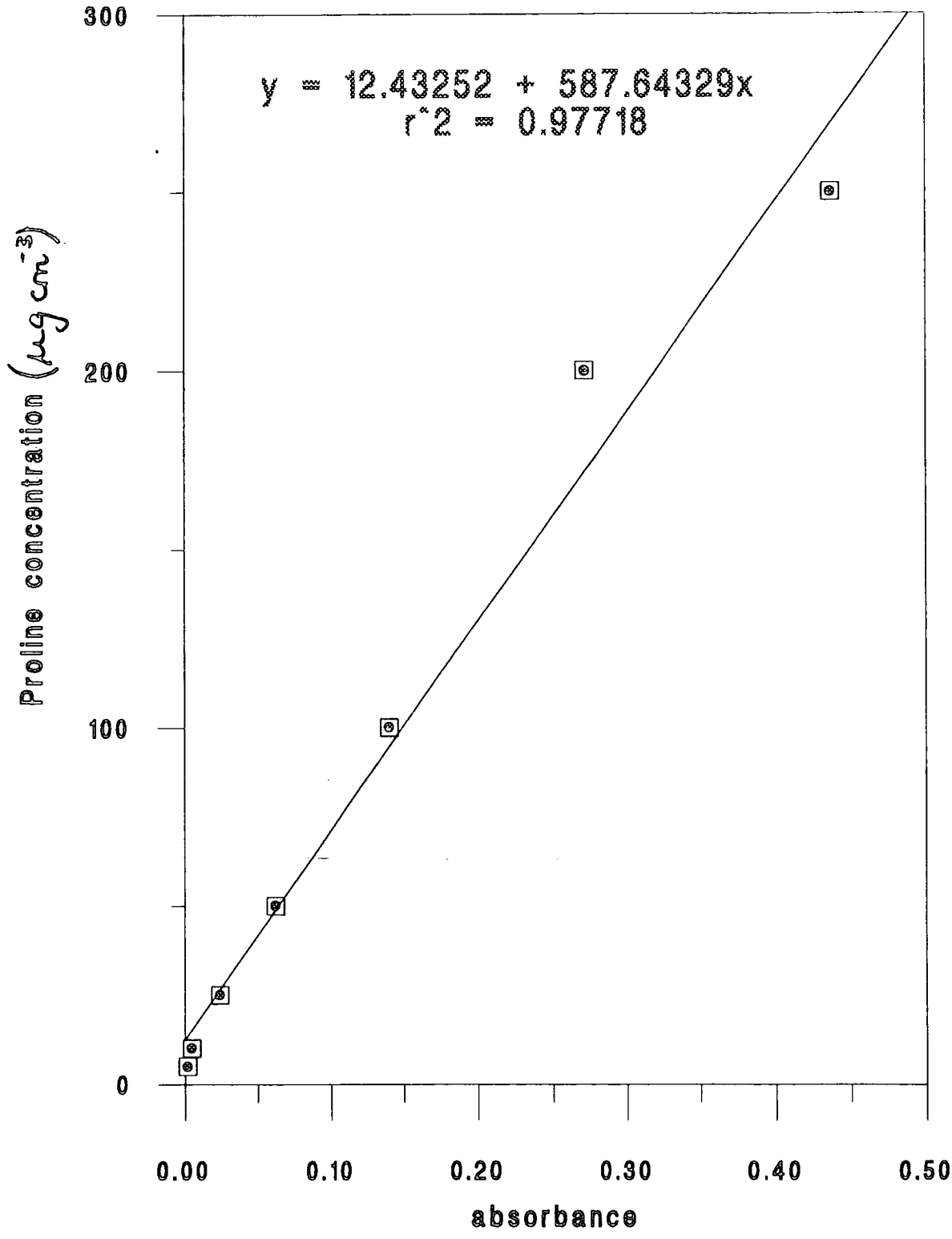

Table 3.4.1.i.

Largest leaf data for plants in four groups.

Group A			Group B		
L	W	L/W	L	W	L/W
33	6	5.50	75	10	7.50
34	6	5.67	131	12	10.
45	9	5.00	116	13	8.92
41	8	5.13	193	14	13.79
38	6	6.33	102	11	9.27
45	9	5.00	118	12	9.83
34	5	6.80	131	13	10.08
33	7	4.71	203	12	16.92
35	4	8.75	103	11	9.36
32	5	6.40	104	12	8.67

Group C			Group D		
L	W	L/W	L	W	L/W
50	13	3.85	112	21	5.33
47	7	6.71	88	12	7.33
44	10	4.40	135	11	12.27
66	12	5.50	75	11	6.82
55	14	3.93	185	16	11.56
60	11	5.45	138	11	12.55
54	16	3.38	103	15	6.87
62	12	5.17	107	12	8.92
62	12	5.17	160	17	9.41
45	10	4.50	222	14	15.86
			86	12	7.17
			90	16	5.63

L = length
W = width
L/W = ratio of length to width
Group A Slei plants in Slei soil
Group B Barningham plants in Slei soil
Group C Slei plants in garden soil
Group D Barningham plants in garden soil

Table 3.4.2.i

SLW for leaves from plants from four groups

Group A	Group B	Group C	Group D
0.2515	0.2479	0.2747	0.2382
0.2471	0.2311	0.2769	0.3724
0.2652	0.2201	0.2816	0.2543
0.2721	0.1940	0.2809	0.2878
0.2864	0.2336	0.3110	0.2430
0.3094	0.2315	0.2519	0.2456
0.2689	0.2335	0.3321	0.1899
0.2672	0.2441	0.2812	0.2349
0.2962	0.2678	0.3483	0.2293
0.3021	0.2166	0.2595	0.2098
0.3401	0.1990	0.2467	0.1846
0.2720	0.2228	0.2602	0.1765
0.2897	0.2296	0.3237	0.1988
0.3653	0.2485	0.3757	0.2534
0.2446	0.2743	0.2657	0.2050
0.3634	0.2455	0.2950	0.2432
0.2718	0.3201	0.2500	0.2064
0.2745	0.2311	0.2629	0.2814
0.3496	0.2274	0.2873	0.1822
0.2933	0.2101	0.2718	0.2387
0.3128	0.2560	0.3064	0.2424
0.3255	0.3397	0.3211	0.2774
0.3159	0.2466	0.2535	0.2136
0.2617	0.1902	0.2426	0.2815
0.2887	0.2460	0.2035	0.2340
0.3636	0.2533	0.2327	0.2962
0.3097	0.2670	0.2487	0.2378
0.3442	0.2079	0.2821	0.2608
0.2789	0.2809	0.2990	0.2592
0.3048	0.2949	0.2160	0.2876

Group A Slei plants in Slei soil
Group B Barningham plants in Slei soil
Group C Slei plants in garden soil
Group D Barningham plants in garden soil

Table 3.4.2.i.
Numbers of leaves with and without petioles.

Group D			Group C			Group B		
a	b	c	a	b	c	a	b	c
5	4	9	0	8	8	2	5	7
5	2	7	0	9	9	3	3	6
3	1	4	0	9	9	3	3	6
4	1	5	3	6	9	6	1	7
5	1	6	1	4	5	6	4	10
4	2	6	0	6	6	4	2	6
4	3	7	1	6	7	6	3	9
5	3	8	0	9	9	5	2	7
7	2	9	1	8	9	5	2	7
6	3	9	4	8	12	4	2	6
6	3	9						
5	4	9						

Group A Slei plants in Slei soil
Group B Barningham plants in Slei soil
Group C Slei plants in garden soil
Group D Barningham plants in garden soil

a number of leaves with petioles
b number of leaves without petioles
c total number of leaves

Table 3.5.3.i

Wts (mg) and areas (mm^2) of leaves offered (W.O. and A.O.), and area eaten (A.E.) in palatability tests

	Snail	W.O.	A.O.	A.E.	W.O.	A.O.	A.E
Test 1		Group A			Group C		
	1	60.2	177	62	48.1	195	68
	2	32.1	118	1	52.3	201	11
	3	42.0	145	0	49.2	152	0
	4	36.9	101	1	43.2	115	115
	5	42.8	175	0	64.3	242	0
	6	62.5	172	0	71.1	241	0
	7	23.1	85	85	39.0	156	102
	8	40.9	149	149	39.7	151	34
	9	41.6	119	1	72.1	251	71
	10	83.0	283	0	76.1	280	3
Test 2		Group A			Group D		
	11	50.8	202	142	46.7	253	131
	12	42.5	172	0	28.6	162	49
	13	43.5	164	4	32.2	162	162
	14	40.0	147	0	40.8	161	161
	15	71.6	250	84	49.0	239	33
	16	72.7	235	0	62.5	257	237
	17	58.9	219	51	48.5	235	235
	18	46.5	174	0	87.5	311	73
	19	46.5	157	0	30.8	169	53
	20	86.7	287	5	67.3	282	94
Test 3		Group D			Group B		
	1	140.3	589	316	60.7	305	246
	2	175.4	471	336	93.8	421	0
	3	158.4	623	403	85.2	371	300
	4	132.4	460	0	108.6	437	258
	5	186.9	769	7	183.5	669	10
	6	101.2	412	0	87.9	358	211
	7	114.5	603	27	97.0	303	289
	8	113.7	484	179	89.2	386	23
	9	110.5	482	5	83.0	365	150
	10	126.5	603	4	89.5	426	76
Test 4		Group C			Group B		
	11	130.5	475	0	101.9	411	102
	12	93.6	338	160	94.3	408	45
	13	105.3	374	0	91.8	417	321
	14	102.8	366	13	52.0	268	43
	15	113.5	365	95	100.7	431	125
	16	115.1	457	0	81.5	352	284
	17	196.3	591	345	80.8	346	49
	18	80.7	287	0	171.6	703	0
	19	132.0	379	0	127.2	475	0
	20	115.2	444	393	101.6	469	191

Table 3.5.3.i cont.

Wts (mg) and areas (mm^2) of leaves offered (W.O. and A.O.), and area eaten (A.E.) in palatability tests

	Snail	W.O.	A.O.	A.E.	W.O.	A.O.	A.E.
Test 5		Group C			Group D		
	1	91.6	299	77	141.3	583	0
	2	146.1	455	455	143.4	517	517
	3	103.7	409	165	138.6	649	358
	4	96.3	397	0	131.2	466	404
	5	69.6	342	0	123.3	527	0
	6	91.0	391	0	107.8	364	133
	7	105.2	423	423	119.6	503	503
	8	107.2	380	0	101.7	390	38
	9	94.2	315	0	134.0	517	218
	10	75.8	351	0	188.4	655	124
Test 6		Group A			Group B		
	11	24.4	78	29	25.6	100	100
	12	71.6	220	3	78.8	232	232
	13	33.8	107	0	43.9	178	0
	14	21.2	81	81	23.2	122	122
	15	51.1	177	0	42.8	174	79
	16	28.0	77	50	42.3	167	167
	17	38.4	124	0	61.4	230	0
	18	32.7	95	0	31.6	152	0
	19	26.5	95	20	44.1	157	157
	20	31.7	104	26	28.9	98	98
Test 7		Group D			Group D		
	1	70.4	281	0	83.0	275	57
	2	120.8	448	8	121.6	451	0
	3	148.7	586	0	164.3	598	15
	4	82.2	292	28	61.4	236	104
	5	88.0	335	0	104.4	405	0
	6	91.8	354	0	83.8	317	0
	7	91.5	381	252	89.7	359	271
	8	98.2	302	92	88.2	367	42
	9	87.3	354	157	101.8	373	41
	10	65.6	273	6	66.8	288	0
Test 8		Group D			Group D		
	11	63.3	269	0	74.1	309	4
	12	69.8	289	3	70.8	278	0
	13	77.2	326	0	73.9	300	1
	14	46.7	205	12	49.5	212	27
	15	68.6	275	2	73.8	314	0
	16	52.4	226	216	41.3	176	157
	17	59.7	228	0	62.9	255	0
	18	63.8	228	0	66.8	238	0
	19	65.7	249	249	72.5	277	277
	20	40.8	143	96	41.8	186	20

Table 3.5.3.ii

Weight in mg eaten by each snail in tests 1 - 8.

	Test 1		Test 2		Test 3	
sn	A	C	D	B	C	D
1	21.09	16.77	75.27	48.96	23.59	0.00
2	0.27	2.86	125.13	0.00	146.10	143.40
3	0.00	0.00	0.00	68.89	41.83	76.45
4	0.37	43.20	0.00	64.12	0.00	113.74
5	0.00	0.00	1.70	2.74	0.00	0.00
6	0.00	0.00	0.00	51.81	0.00	39.39
7	23.10	25.50	5.13	92.52	105.20	119.60
8	40.90	8.94	42.05	5.32	0.00	9.91
9	0.35	20.39	1.15	34.11	0.00	56.50
10	0.00	0.82	0.84	15.97	0.00	35.67

	Test 4		Test 5		Test 6	
	A	D	C	B	A	B
11	35.71	24.18	0.00	25.29	9.07	25.60
12	0.00	8.65	44.31	10.40	0.98	78.80
13	1.06	32.20	0.00	70.67	0.00	0.00
14	0.00	40.80	3.65	8.34	21.20	23.20
15	24.06	6.77	29.54	29.21	0.00	19.43
16	0.00	57.64	0.00	65.76	18.18	42.30
17	13.72	48.50	114.59	11.44	0.00	0.00
18	0.00	20.54	0.00	0.00	0.00	0.00
19	0.00	9.66	0.00	0.00	5.58	44.10
20	1.51	22.43	101.97	41.38	7.92	28.90

	Test 7			Test 8	
sn	D	D	sn	D	D
1	0.00	17.20	11	0.00	0.96
2	2.16	0.00	12	0.72	0.00
3	0.00	4.12	13	0.00	0.25
4	7.88	27.06	14	2.73	6.30
5	0.00	0.00	15	0.50	0.00
6	0.00	0.00	16	50.08	36.84
7	60.52	67.71	17	0.00	0.00
8	29.92	10.09	18	0.00	0.00
9	38.72	11.19	19	65.70	72.50
10	1.44	0.00	20	27.39	4.49

Group A Slei plants in Slei soil
Group B Barningham plants in Slei soil
Group C Slei plants in garden soil
Group D Barningham plants in garden soil

Table 3.5.3.iii

Palatability Indices in all tests.

	Test 1		Test 2		Test 3	
sn	A	C	D	B	C	D
1	0.56	0.44	0.61	0.39	1.00	0.00
2	0.09	0.91	1.00	0.00	0.50	0.50
3	0.00	0.00	0.00	1.00	0.35	0.65
4	0.01	0.99	0.00	1.00	0.00	1.00
5	0.00	0.00	0.38	0.62	0.00	0.00
6	0.00	0.00	0.00	1.00	0.00	1.00
7	0.48	0.52	0.05	0.95	0.46*	0.53*
8	0.82	0.18	0.89	0.11	0.00	1.00
9	0.02	0.98	0.03	0.97	0.00	1.00
10	0.00	1.00	0.05	0.95	0.00	1.00

	Test 4		Test 5		Test 6	
sn	A	D	C	B	A	B
11	0.60	0.40	0.00	1.00	0.26	0.74
12	0.00	1.00	0.81	0.19	0.01	0.99
13	0.03	0.97	0.00	1.00	0.00	0.00
14	0.00	1.00	0.30	0.70	0.48	0.52
15	0.78	0.22	0.50	0.50	0.00	1.00
16	0.00	1.00	0.00	1.00	0.30	0.70
17	0.22	0.78	0.91	0.09	0.00	0.00
18	0.00	1.00	0.00	0.00	0.00	0.00
19	0.00	1.00	0.00	0.00	0.11	0.89
20	0.06	0.94	0.71	0.29	0.22	0.78

	Test 7			Test 8	
sn	D	D	sn	D	D
1	0.00	1.00	11	0.00	1.00
2	1.00	0.00	12	1.00	0.00
3	0.00	1.00	13	0.00	1.00
4	0.23	0.77	14	0.30	0.70
5	0.00	0.00	15	1.00	0.00
6	0.00	0.00	16	0.58	0.42
7	0.47	0.53	17	0.00	0.00
8	0.75	0.25	18	0.00	0.00
9	0.78	0.22	19	0.48	0.52
10	1.00	0.00	20	0.86	0.14

Group A Slei plants in Slei soil
Group B Barningham plants in Slei soil
Group C Slei plants in garden soil
Group D Barningham plants in garden soil