HGCA

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DEVELOPMENT AND EVALUATION OF A TECHNIQUE FOR THE RAPID MEASUREMENT OF CEREAL ROOT SYSTEMS

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DEVELOPMENT AND EVALUATION OF A TECHNIQUE FOR THE RAPID MEASUREMENT OF CEREAL ROOT SYSTEMS

by

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ABSTRACT

More rapid, and less expensive techniques for assessing cereal root systems in the field would assist greatly in the development of effective root management strategies. The aim of management is to manipulate the root system to overcome potential limitations on the canopy imposed by adverse soil conditions. Research was undertaken to evaluate a new technique, which has the potential for faster data capture in the field and which avoids the need for time-consuming washing of root samples. The proposed technique involves the use of an air-knife and digital photography. The air-knife delivers a stream of air at high velocity, which enters the pore spaces in soil aggregates causing them to disintegrate. The principle of the proposed technique is to dig a soil-trench within the crop, excavate a known volume of soil from the trench (profile) wall with the air-knife, photograph the exposed roots using a digital camera and measure the length of roots by image analysis. The main objectives of the research were to:

a) establish whether photographs taken of cereal roots in the field provide sufficient resolution for image analysis
b) establish whether the air-knife is a suitable method of soil excavation for reliable determination of cereal root length.

Most experiments were conducted on spring barley crops at, or shortly after, anthesis. The results show that using a high quality camera, digital photographs taken of roots on a profile wall can provide sufficient resolution for image analysis. The resolution was not affected significantly by soil type in the narrow range of soil types investigated. Excavation of soil from the profile wall using the air-knife led to a considerable loss of roots and as such did not provide a reliable estimate of root length density (root length per unit volume of soil). However, the air-knife can be used to expose roots in order to photograph and analyse their number and distribution. When used for this purpose, the technique offers significant savings in time and cost over standard profile wall methods. In particular, it is estimated that the time spent in the field can be reduced by up to 70%.

The technique can help reduce the cost of measurements in future HGCA-funded research, where information is needed on root depth and distribution. This might include variety evaluations or investigations of the effects of soil management practices on root distribution. Where assessments of root length density are required, the technique would need to be calibrated against, or replaced by, soil coring. Digital photographs taken of roots exposed on a profile wall can be used to build an image library for use in advisory publications. Photographs, representing a range of known root length densities, could help farmers make semi-quantitative assessments of the state of rooting of their own crops.

SUMMARY

Recent developments in understanding the growth and function of wheat canopies, has led to significant improvements in crop management. In comparison, our understanding of cereal root systems and our ability to manage them is poor. A major reason for this is that quantitative measurements of root systems are labour intensive and expensive to make, and are thus rarely undertaken in studies of agronomy or crop physiology. Development of more rapid techniques for quantifying the size and distribution of cereal root systems in the field would enable these measurements to be made more routinely. As such they would help underpin current and future HGCA-funded research on root and soil management.

At present, two of the most widely used techniques for measuring root systems are soil coring and profile wall methods. Soil coring is the standard for determining root length density (length of roots per unit soil volume). It requires relatively little time spent in the field, but involves time-consuming laboratory procedures to wash roots from the soil, remove contaminating debris, and spread the root system prior to measurement. Profile wall measurements, on the other hand, involve digging a trench in the field and counting, mapping or estimating the length of roots on an exposed wall of the trench. Overall, profile wall methods can be quicker than soil coring, but require more time in the field and do not provide direct measurements of root length density.

A new technique has been proposed, involving the use of an air-knife and digital photography, which could combine the advantages of soil coring with those of the profile wall method. The air-knife is used to excavate soil. It works by generating a stream of high velocity air, which enters the pore spaces in soil aggregates causing them to disintegrate. The principle of the proposed technique is to dig a soil-trench within the crop, excavate a known volume of soil from the trench wall with the air-knife, photograph the exposed roots using a digital camera, and measure the length of roots in the captured images by image analysis. Research was undertaken to evaluate the proposed technique for measuring cereal root systems. The specific objectives were:

- to establish whether digital photography in the field can provide sufficient resolution for image analysis
- to determine whether the technique provides reliable quantitative data on root length and distribution compared to standard methods
- to investigate the effects of a limited range of soil types on the ease of soil excavation and reliability of root length measurements
- to conduct an analysis of the time required to operate the technique in comparison to standard methods.

Most experiments were conducted on spring barley crops at, or shortly after, anthesis. Soil trenches were dug manually and a 20 x 20 cm grid marked on the profile wall by hammering bolts into the wall at a

spacing of 10 cm. The bolts served as both a reference framework for aligning and calibrating photographs, and as depth gauges for estimating the depth of excavation. The camera (a Nikon Coolpix 990) was lowered into the trench on a purpose built jig. Root lengths on the digital images were measured by first tracing over the roots using a pure white ‘paintbrush’ in the image software package Photoshop 5.5 (Adobe Systems Inc) and analysing the tracings in the image analysis software WinRhizo (Régent Instruments Inc., Quebec). Similarly roots could be counted by marking them at one end with the ‘paintbrush’ and counting the marks in WinRhizo.

The results show that digital photographs taken of cereal roots, using a high quality camera, can provide sufficient resolution for image analysis. Most roots present on the profile wall were visible in single images of the entire 20 x 20 cm grid. Increasing the image resolution four-fold resulted in only a 6% increase in total root length measured in the image. Automatic and semi-automatic analyses of the images using WinRhizo, without prior tracing of the roots, gave unreliable estimates of their length. This was because of the variable colour and brightness of the profile wall in the photograph and its poor contrast with the roots. The scale of the error differed with soil type and was greatest with the sandy silt loam and least with the loamy sand and sandy loam. Soil type had no appreciable effect on the resolution of the captured images and the ability to identify roots for manual tracing. Tracing and marking of roots was quick; 10 minutes for tracing roots in an image of a 20 x 20 cm profile wall grid, and 4 minutes for marking and counting.

The precision of excavation using the air-knife was determined by assessing the variation in depth of soil removed across the profile. The precision differed with site (soil type) and was least at sites with the greatest soil strength and stone content.

The effect of excavation by the air-knife on the extent of root loss was determined by excavating to different pre-determined depths. After each depth, a photograph was taken of the exposed roots before the next depth increment was excavated. The root length measured in the photographs remained relatively constant in spite of the increasing depth of excavation. The root length density (RLD) was estimated from the root length measured in the photograph and the total volume of soil removed from the profile wall, and was compared with that determined by soil coring. RLD (as a % of that determined by soil coring) ranged from 40% at the shallowest excavation (0.75 cm) to only 7% at the deepest (4.25 cm). The results indicate that extent of root loss increased with increasing depth of excavation. In an additional experiment on mixed grassland, conducted in mid September, mesh screening was used to cover the profile wall during excavation with the air-knife. The hypothesis tested was that reducing the movement of soil particles during excavation would reduce the extent of root loss. Soil was trapped behind the screen after excavation, indicating that movement had been reduced, but it had no effect on the scale of root loss from the profile wall. The results demonstrate that the air-knife cannot be used to excavate soil for direct estimates of root length density at the profile wall.

In conclusion, the study has demonstrated that digital photography can be used successfully in the field to record the roots exposed on a profile wall. Although the length of roots measured from these images does not give a reliable estimate of the actual root length density, the images do provide valuable information on relative root abundance, distribution and rooting depth. Where this type of information is needed, air-knife excavation and digital photography offer significant advantages over standard profile wall methods. The technique greatly increases the rate of data capture and it has been estimated that the time spent in the field can be reduced by as much as 70%. If root number, rather than root length, is quantified, analysis of images can be completed quickly, and a total time requirement for collecting and analysing data from a 1 x 1 m profile wall is estimated to be ca 100 minutes. This represents a saving of about a third over previously reported techniques. Air-knife excavation and digital photography also offer advantages in terms of increased operator comfort and safety.

The technique can help reduce the cost of measurements in future HGCA-funded research, where information is needed on root depth and distribution. This might include variety evaluations or investigations of the effects of soil management practices on root distribution. Where assessments of root length density are required, the technique would need to be calibrated against soil coring. Digital photographs taken of roots exposed on a profile wall can be used to build an image library for use in advisory publications. When farmers dig soil pits to inspect soil conditions, they are looking at a profile wall. Reference photographs of roots on profile walls, representing a range of known root length densities, could help farmers make semi-quantitative assessments of the root system of their own crops.

1. INTRODUCTION

Recent developments in understanding the growth and function of wheat canopies has led to improved guidelines for crop management (Anon 1997). Decisions regarding sowing date, seed rate, variety, N inputs and fungicide applications can be made more flexibly and with greater confidence, because they are based on a better understanding of crop function. In comparison, our knowledge of cereal root systems, and how to manage them, is less well developed, and yet adverse soil conditions which restrict root growth and activity can lead to significant yield losses (Belford *et al*. 1985, Foulkes *et al*. 1998). The size and distribution of a root system required to support the canopy under a particular set of conditions is generally not known.

The major reason for our current poor understanding of root systems is that quantitative assessments are difficult, time-consuming and expensive to make. Consequently, measurements of root systems are rarely undertaken in studies of crop growth. More rapid and less expensive techniques for assessing root systems in the field would assist greatly in the development of effective root management strategies.

The following information is required for research and advisory purposes:

- a quantitative assessment of root length density (length of roots per unit volume of soil) with depth down the soil profile
- a measure of root distribution including the degree of clustering of roots along cracks or pores within particular soil horizons
- a visual record of the size and distribution of the root system.

In order to provide advice to farmers on when and where root management will improve yield, research is needed to determine the size and distribution of a root system necessary to support the canopy under a particular set of conditions. This will require *quantitative* measurements of root systems. Clustering of roots can lead to localized deficiencies of water and nutrients and should also be recorded in an assessment of root distribution in this research. Farmers are unlikely to have the time or facilities to undertake quantitative assessments of root systems. A photographic library illustrating adequate and inadequate root system size and distribution for given soil conditions will enable farmers to make semi-quantitative, visual assessments of their own crops. A system, similar to the guides for assessing disease severity on leaves, might be developed. A technique which incorporates all three types of information, and which is relatively rapid, would enable root systems to be measured more routinely.

1.1 Current methods of root sampling

At present there are a range of techniques available for sampling and measuring root systems in the field. Each has its merits and limitations (Mackie Dawson and Atkinson 1991; Oliveira *et al*. 2000; van Noordwijk *et al*. 2000). The most widely used techniques are outlined below:

1.1.1 Extraction methods

These involve the excavation of soil and roots. As such they are invasive and non-repeatable. They require a minimum of specialised equipment and may be performed after only a short period of training.

a) *Soil coring*. This is often considered the benchmark technique for measuring root length density. It provides a quantitative estimate, of root length down the profile. After extraction from the ground, cores are cut into sections, soil washed from the roots, and organic debris such as straw and dead root material removed by hand before root lengths are measured. The washing and clean-up stages are the most time-consuming part of the process. Using the latest image analysis software, length measurements can be made reasonably quickly, but spreading of the root system to avoid overlaps still requires a considerable manual effort (see 1.2 below). Roots washed from soil cores often have to be sub-sampled (by weight) to provide a size of sample that can be measured easily. Estimates of root lengths then have to be corrected to take into account the sub-sampling. Alternatively all the sub-samples can be measured separately and the lengths summed. One advantage of soil coring is that the sampling time in the field is relatively short.

b) *Profile wall methods*. In this technique a trench is dug, the wall of the trench is smoothed with a profiling knife or trowel and gently washed. Counts of exposed roots and a record of their location are then made. This often involves tracing the point of emergence of a root on an acetate sheet that can be digitized at a later date. If required, root counts can be converted to estimates of root length density using empirically derived calibrations or theoretical considerations of root orientation (van Noordwijk *et al*. 2000). It is a useful method for determining the distribution and clustering of roots, but has the disadvantage that it requires a long time to be spent in the field, and good weather conditions.

1.1.2 Non-destructive methods

1. These techniques employ various invasive or non-invasive methods to provide repeatable estimates of root growth. The data collection procedures are generally rapid, but require specialized and costly equipment. Data processing is time-consuming and requires expert interpretation. The most widely used non-destructive techniques are the “glass wall” techniques. Here a transparent viewing surface is

inserted into the soil. The earliest form of this was a window or whole wall of an underground room (rhizotron). These have now been largely superseded by Perspex tubes (minirhizotrons) with a diameter of several centimetres, which are much less expensive and are portable. In both versions a camera system is used to record images of roots that come into contact with the transparent surface. Its main application is for determining rates of root production and mortality. It is less useful for measuring root length as it has to be calibrated against another technique such as soil coring.

1.2 Determination of root length

1.2.1 Manual methods

A variety of techniques are available for determination of root length in washed samples, or on tracings from profile walls (Oliveira *et al*. 2000). Direct measurement is impractical for all but the smallest samples. The line-intersection method (Tennant 1975) is the most widely used manual technique for indirectly *estimating* root length. Roots are spread over a grid whose unit grid size is known (e.g. 1.0 or 0.5 cm). The roots must be separated to minimize overlap and orientated randomly on the grid. The total number of intersections between roots and the horizontal and vertical grid lines are then counted and used to estimate the root length (Tennant 1975; Oliveira *et al*. 2000). Errors in the estimates may arise from several sources; the orientation of roots, poor contrast of roots with their background, the definition of an intersection and variation between operators.

The technique can be very time-consuming and tedious, depending on the size of the root sample to be measured. The most laborious part of the procedure is the spreading of roots, rather than the counting of line-intersections. The separation of roots is made easier by placing them in a shallow layer of water (2-3 mm).

1.2.2 Image analysis

Measurements of roots using computer-based image analysis are more rapid, more accurate and potentially less susceptible to human error than manual methods. In addition, they can provide information on morphological characteristics such as diameter and branching pattern.

Image analysis is used most extensively with washed root samples (e.g. from soil cores) and recordings from mini rhizotrons. Preparation of washed samples is similar to that for the line-intersection method. Samples need to be cleaned up to remove as much contaminating material as possible and spread out in a tray. Although some image analysis systems are able to accommodate a certain amount of extraneous material and root overlap, cleaning and spreading of samples is still the most time-consuming part of the measurement process.

Images of washed roots are, for the most part, recorded using electronic cameras or scanners. Although coloured images can be recorded, most image analysis systems utilize grey-scale information, because the file sizes are smaller.

The spatial resolution of the camera or scanner sets a limit on the minimum size of root that can be analysed. The spatial resolution is the number of pixels recorded per unit area. Ideally, the thinnest roots should have a diameter of at least 3 pixels to enable accurate determination of diameter and to allow for thresholding (Richner *et al*. 2000). A value of brightness is associated with each pixel in an image. For grey-scale images this is often in the range 0 – 255 (8-bit grey scale images). The maximum number of brightness values that can be recorded by a camera or scanner is known as its radiometric resolution (Richner *et al*. 2000). Differences in brightness value forms the basis for segmenting (separating) roots from their background, and any extraneous objects, by grey-level thresholding. The technique involves setting a grey-level value above which pixels will be classified as belonging to roots and below which, they will be excluded as non-root objects.

1.3 Areas for improvement

More rapid and lower cost measurements of root systems may be achieved through improvements in both root sampling techniques and the efficiency of data capture/processing. In particular, development of techniques which reduce the time spent on data capture in the field and the need for extensive clean-up and spreading of root samples, would be beneficial.

A new technique is proposed, which has the potential to combine the advantages of soil coring with those of the profile wall method, but which is more rapid. It involves the use of an air-knife. The air-knife delivers a stream of air at a speed of Mach 2, which enters the pore spaces in soil aggregates causing them to disintegrate. The air-knife has been used successfully to excavate tree root systems, leaving medium to fine roots intact (Rizzo and Gross 2000). The principle of the proposed technique is as follows. A trench is dug and the wall of the trench excavated to a known depth using the air-knife. The exposed roots are then photographed using a digital camera and the root length density determined from the image by image analysis.

The technique has the potential to provide a rapid quantitative, or semi-quantitative, measurement of root length density, distribution and degree of clustering, plus a visual record for archiving in an image library. The library could then be used to provide photographs for advisory publications illustrating specific features of the root system, or to help farmers assess, semi-quantitatively, the root length and distribution of their own crops.

The purpose of this project is to conduct a pilot study to evaluate the technique for measuring cereal root systems. In particular, development work is needed to:

a) Establish whether photographs taken of cereal roots in the field provide sufficient resolution for image analysis.
b) Establish whether the air-knife is a suitable method of soil excavation for reliable determination of cereal root length.

2. AIMS & OBJECTIVES

2.1 Overall Aim

To evaluate the potential of a new technique based on the use of an air-knife and digital camera for the rapid quantitative assessment of root systems in the field.

2.2 Specific Objectives:

- To establish whether the technique can provide sufficient resolution for image analysis.
- To determine whether the technique provides reliable quantitative data on root length and distribution compared to standard methods.
- To investigate the effects of a limited range of soil types on ease of root excavation and reliability of root length measurements.
- To conduct an analysis of the time required to operate the technique in comparison to standard methods.

3. MATERIALS & METHODS

Three field experiments were conducted in the summer of 2000 to evaluate different aspects of the technique.

- *Experiment 1*: Effects of soil texture on the ease of excavation by the air knife and on the image quality for root length determination. An analysis was made of the accuracy of measuring root length from digital photographs using automatic, semi-automatic and manual methods of image processing.

- *Experiment 2*: Effects of depth of air-knife excavation on the estimates of root length density. Profile walls were excavated to progressively increasing depth. At each depth, photographs were taken for analysis of root length and estimates of root length density compared to those derived from standard soil coring.

- *Experiment 3*: An investigation of the possible benefits of using mesh screening to cover the profile face during air-knife excavation. Profile walls were excavated to a prescribed depth with and without a screen of mesh. Soil and root tissue was collected from different locations within the soil pit to determine the root losses resulting from the excavation.

3.1 Location of field sites

Experimental sites (Tables 1 and 2) were selected to provide a range of soil textures. Experiments 1 and 2 were carried out on commercial spring barley crops during July. At the time field measurements were made, the barley crops were at Zadoks growth stage 61-83, depending on the site. Experiment 3 was conducted on mixed grassland in mid September. The anatomy and morphology of grass root systems are comparable with those of cereals. Use of grassland allowed the experimental period to be extended into September when the spring barley crops were close to harvest and their root systems senescing. The experiments conducted at each site are listed in Table 1.

Table 1. *Location of field sites*

Site	Location	Experiment
S1	Gladhill farm, Garmouth, Morayshire	1
S2	SAC-Aberdeen, Sunnybrae farm, Bucksburn, Aberdeen	1 & 2
S3	SAC-Aberdeen, Tillycorthie farm, Udny, Aberdeenshire	1
S4	SAC-Aberdeen, Craibstone Estate, Bucksburn, Aberdeen	3

Table 2. *Site details*

	S1	S2	S3	S4
OS ref	NJ 322651	NJ 876115	NJ 912238	NJ 865110
Soil series	Boyndie	Terryvale	Pitmeddon	Corby
Soil association	Boyndie	Countesswells	Tarves	Corby
Top soil texture	Loamy sand	Sandy loam	Sandy silt loam	Stony sandy loam
Crop	S Barley	S Barley	S Barley	Mixed grassland

3.2 Excavation of soil pits

Soil pits approximately 40 cm deep and 60 cm square were dug manually. A profile wall, which ran across the crop drills, was selected for excavation and measurement. Where possible a wall out of direct sunlight was chosen, to facilitate flashlight photography. After digging, the selected wall was smoothed with a trowel. Nine 6 mm diameter bolts were then driven, at a spacing of 10 cm, into the soil face with a hammer to form a grid lattice 20 x 20 cm, with the head of each bolt flush with the soil (Fig. 1). The upper edge of the grid was located 5 cm below the soil surface. The grid provided a reference framework for photographing the exposed roots and for calibrating the image analysis. The bolts were marked in increments of 0.5 cm along their length and served as depth gauges so that the depth of excavation could be estimated. To ensure uniformity between replicate soil pits and sites, a template was used to lay out the grid.

The air-knife (or soil-pick; MBW Ltd, Bolton, Lancs.) was coupled to an air compressor capable of delivering 59 l s^{-1}. Soil was excavated steadily from the profile wall to the prescribed depth, with smooth side to side movements of the nozzle. Uniformity of excavation was estimated by recording the exposed length of the bolts at each of the nine points on the grid (Fig. 2).

3.2.1 Variations in excavation according to experiment

Experiment 1. Profile walls were excavated to a target depth of 5 cm and photographs taken (section 3.3). The actual depth of excavation was measured by recording the exposed length of the marker bolts. Roots on the exposed face of the wall were then cut and collected. The roots were placed between two pieces of paper towelling moistened with water and, on return to the laboratory, stored frozen prior to measurement of root length.

Experiment 2. Profile walls were excavated progressively to a target range of 0.5-1.0, 1.0-1.5, 1.5-2.5, 2.5-3.5 and 3.5-5.0 cm. After each excavation, the exposed length of each marker bolt was recorded and the profile wall photographed. In another set of pits, the profile wall was excavated manually using a bricklayer’s trowel to a depth of 0.5 cm and then photographed.

Experiment 3. Profile walls were smoothed and covered with 4 mm gauge nylon mesh. The mesh was pinned in place using fencing staples. The grid of depth-marking bolts was set up by driving the bolts into the soil through holes in the nylon mesh. The floor of the pit was covered with plastic sheeting, one edge of which was pinned to the profile wall. Soil was excavated from the pit wall using the air-knife through the mesh to a depth of 3 cm. At intervals during the excavation, the soil and any root tissue that came through the mesh was collected from the plastic sheeting and retained in a polythene bag. Similarly, the loose soil trapped behind the mesh was recovered and retained. The mesh was then pushed back against the profile wall and the excavation continued until the target depth was reached. After the excavation had been completed and all the soil recovered, the mesh was removed, taking care not to dislodge the marking bolts. The profile wall was photographed. Exposed roots were then cut, allowed to fall on to the plastic sheet on the floor of the pit, from where they were collected and wrapped in moist paper towelling. All soil and root samples were stored frozen prior to analysis.

The profile wall was then smoothed with a trowel, and excavated to the same target depth without mesh screening to determine the effects of screening on the length of roots retained on the profile wall.

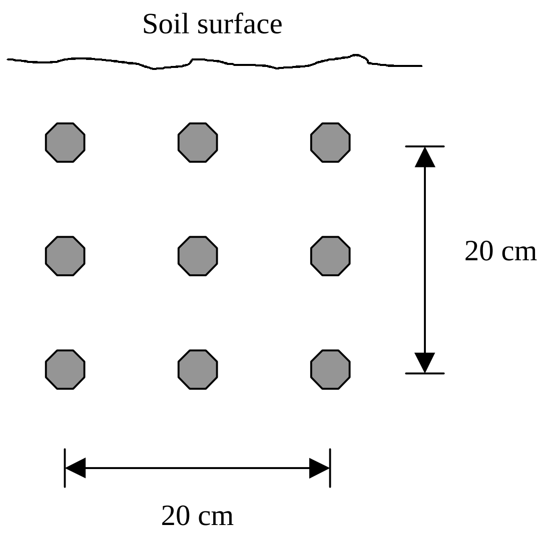

Fig. 1. Arrangement of marking bolts to form 20 x 20 cm grid on the face of the profile wall.

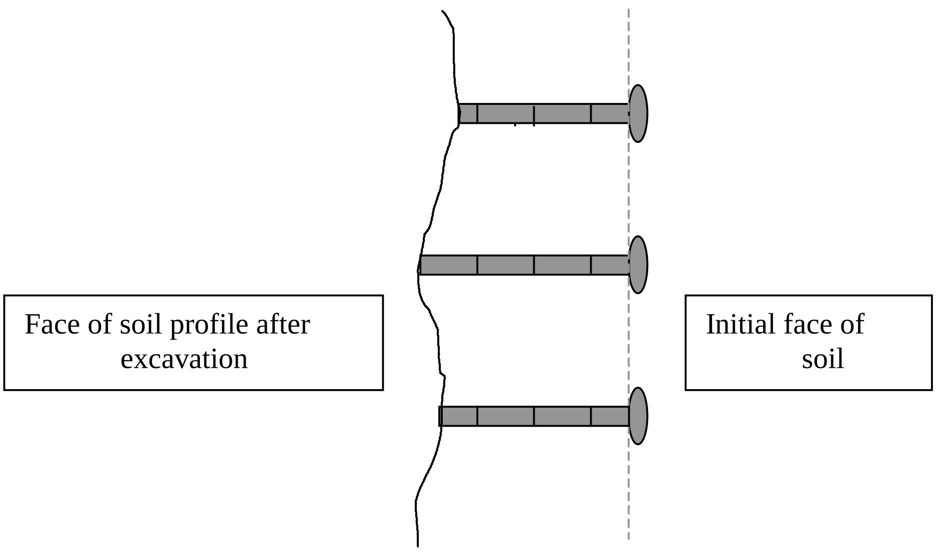

Fig. 2. Diagram of a section through the profile wall to illustrate the use of marking bolts to gauge the depth of excavation.

3.3 Image capture and storage

Roots exposed by the excavations were photographed using a digital camera (Nikon Coolpix 990). The camera was lowered down the pit using a purpose built jig (Fig 3). The jig held the camera perpendicular to the profile face and the Y structured legs provided the required stability on the soil surface. A ratchet system allowed the camera to be lowered to a pre-determined depth from the surface of the ground. Markings on the jig also enabled it to be set at a known distance from the profile wall. These features ensured the same camera position (relative to the ground surface and profile wall) could be located quickly at each site and in each replicate soil pit. Photographs were taken initially of the whole 20 x 20 cm grid, followed in some experiments by zoom images of one or more of the subset 10 x 10 cm frames (Fig. 1).

Preliminary trials were conducted using a range of camera settings under natural and flash lighting to establish a system that would provide the maximum quality of image for subsequent analysis. Details of camera settings used in the experiments are given in section 4.1.1. At the end of each day photographs, were downloaded on to a PC and stored as JPEG files.

3.4 Soil coring

In experiment 2, soil cores were taken to a depth of 30 cm using a 2.5 cm diameter auger. There were four cores per replicate pit; two were taken adjacent to crop plants and two mid-way between the drills. The top and bottom 5 cm lengths of each core were removed and discarded, the remainder was transferred to a polythene bag, frozen and stored at –20^{o}C until analysis. Each sample therefore represented the volume of soil between 5 and 25 cm below the soil surface and corresponded to the profile depth measured on the digital photographs. In experiment 3, eight 2.5 cm cores and four 8.0 cm diameter cores were taken from close to each of the four replicate pits.

In preparation for analysis, soil was washed from the roots under running water. Root tissue was collected over two sieves (5.0 mm and 1.0 mm mesh) and any remaining debris, which included soil particles, grit and organic matter, was removed by hand. The roots were then gently blotted dry and weighed. The whole sample was either spread out in a dish under a thin film of water (2-3 mm), or sub-sampled by weight prior to spreading. A digitized image of the roots was generated using a scanner (Epson 836XL) fitted with a transparency adapter and operating at a resolution of 300 dpi. Root length was determined automatically by batch processing the images using the software WinRhizo version 4.1b (Régent Instruments Inc., Quebec, Canada). Threshold values for pixel inclusion were selected by

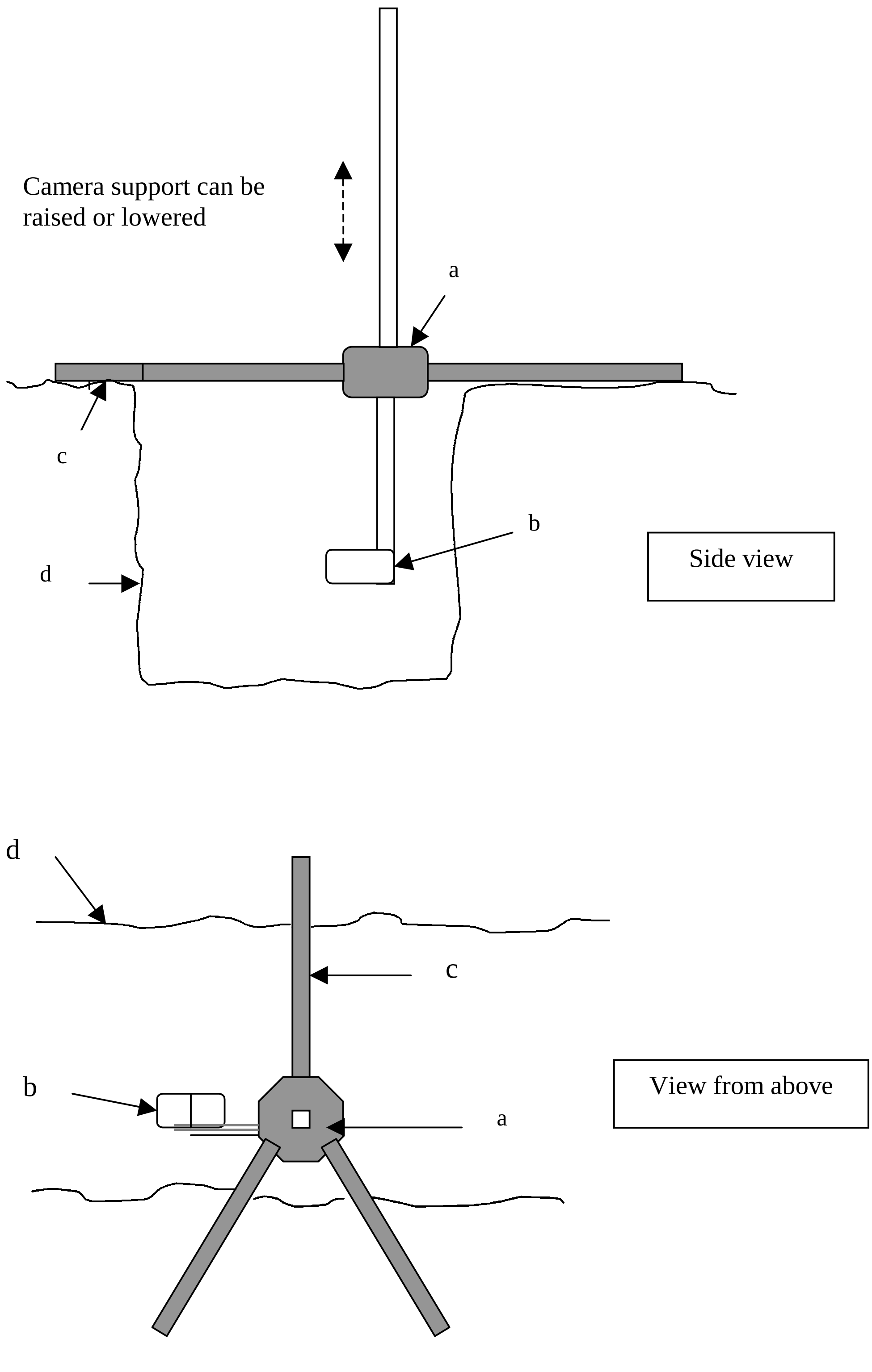

Fig. 3. Diagram of the camera jig. a) ratchet to hold vertical support for the camera; b) camera with viewing screen uppermost; c) supporting legs; d) profile wall of soil pit.

various methods as described below. In addition, items with an area of less than $1mm^2$ were automatically filtered from the analysis. Roots that were cut directly from the profile walls after air-knife excavation were washed and their length determined in the same way.

3.5 Measurement of soil moisture content

After completing the soil excavation, photography and root sampling, soil moisture contents were measured using a theta-probe (Delta T Instruments, UK). In total, four readings were taken from each profile wall; one in each of the four 10 x 10 cm subset frames.

3.6 Timing of operations

All operations involved in the above measurements were timed to the nearest minute so that the efficiency of the technique could be compared to standard methods.

4. RESULTS

4.1 Image capture and analysis

4.1.1 Camera settings

After preliminary trials with different camera settings the following were adopted as standard.

Image size	1,024 x 768 pixels
Image quality	Fine
Exposure	Aperture priority f 7.0, matrix metering
Focus	5-area multi autofocus

The choice of image size was a compromise between the need for high resolution and the number of frames that could be stored on a 32 MB card. When combined with a fine image quality setting, which compressed the file by approximately 1/4, the image size of 1,024 x 768 pixels enabled 80 frames to be stored. The full image size of 2,048 x 1,536 pixels could only be used without compression, and would have meant a storage capacity of just 2 frames per card. As a result, numerous memory cards would have been required to facilitate a day's work in the field, substantially increasing costs and the inconvenience of having to frequently change cards.

Other settings were selected to enable high quality images to be captured remotely with the camera in the soil pit. Aperture priority automatic exposure ensured there was adequate depth of field. Matrix metering, based over 256 segments, provided an appropriate exposure averaged over the whole field of view. Similarly, the 5-area multi autofocus provided extensive coverage of the frame. With the camera located 30 cm away from the profile wall, it was possible to include the whole of the 20 x 20 cm grid in the frame.

A particularly useful feature of the Coolpix 990 is that the viewing screen and lens can be rotated 90^o to each other. This meant that with the camera lowered down into the pit on the jig, the screen could be oriented so that it could be viewed from above. In this way, the picture could be framed by fine adjustment of the jig without having to climb into the pit. With shallow pits it was possible to reach into the hole to press the shutter manually. A time delay between pressing the shutter release and the shutter opening avoided any problems of camera shake. With deeper pits (e.g. 1-2 m) a remote shutter release would be required.

4.1.2 Image processing

Measurements of root length were taken using WinRhizo software. This is an interactive image analysis system specifically designed for root measurements. Most of the operations it performs are automated, but there is, however, one function that can have manual input that is particularly important to the current study. This is the setting of the threshold level for pixel classification as belonging to a root or to part of the background, as described in section 1.2.2. When set to automatic WinRhizo selects one (in "global" mode) or more (in "adaptive" mode) threshold values that are optimized for each image and can take account of local lighting fluctuations. In manual mode the operator selects the threshold value. In this study we used WinRhizo to analyse the same set of images in three different ways as described below. Prior to all analyses the heads of the reference bolts were "painted out" so that they were darker than the roots and as such would be excluded from the analysis.

a. Manual analysis.

In order to be able to compare effects of different operation modes it is necessary to have a baseline from which to work. In this case that baseline is the actual root length visible in the image. To obtain this all roots in an image were traced manually by calling up a pure white 'paintbrush' (intensity = 255) of 2 pixels width in the Photoshop 5.5 package (Adobe Systems Inc.) and following the line of each root. In images of the whole 20 x 20 cm grid the resolution was such that the average root diameter was greater than 2 pixels. Where necessary it was possible to magnify selected parts of the image to highlight details of clustered roots without substantially compromising the accuracy of the tracing (but see below). Images with manual root tracing were then saved as grey-scale uncompressed TIFF files prior to analysis by WinRhizo. An enlarged section of one such image is shown in Fig. 4. Due to the manual tracing of roots these images could be processed using a threshold value of 254, thus excluding all of the image except the root tracings (after painting out of the reference bolts no background points in any image were of this intensity). Counts of numbers of roots can be made in a similar way, by marking each root at its uppermost end using the 'paintbrush' and counting the number of marks in WinRhizo. It would also be possible to construct maps of root distribution from the marked images, although this was not done in the present study.

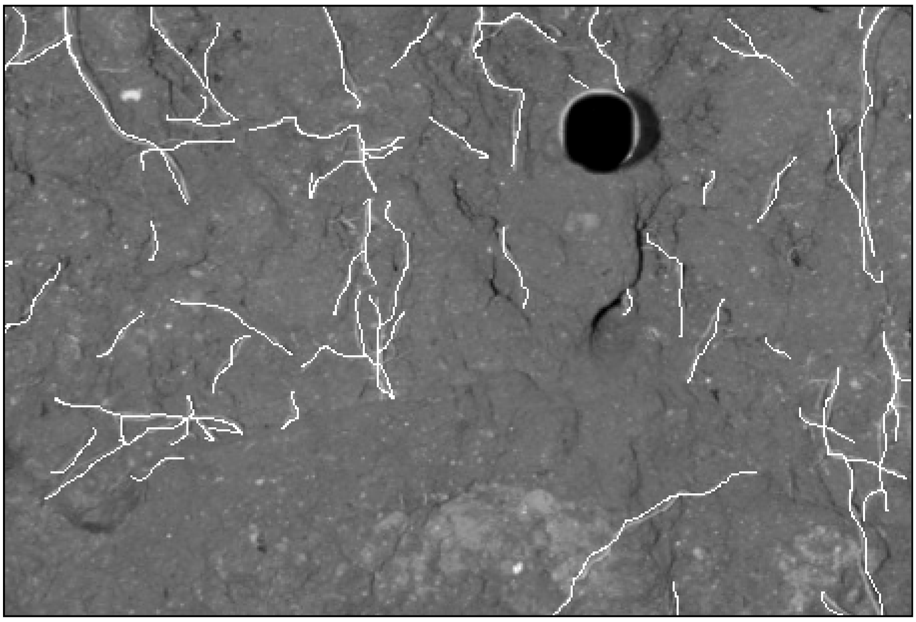

Fig. 4. Close up of one section of a profile wall image showing tracings of roots and a marking bolt ‘painted out’ to exclude it from the analysis.

b. Fully-automatic analysis.

Fully-automatic measurement used the same images as the manual analysis, but without the root tracings (i.e. the image as captured in the field). Image files were converted from JPEG to TIFF format before analysis. WinRhizo was operated in “global automatic threshold” mode in which it selected an optimum value for each image. Fully-automatic measurements greatly overestimated the root length compared to manual tracing (Fig. 5). Based on mean values, the overestimation ranged from 4.6 x to 18.3 x, depending on the site (Fig. 5). The extent of overestimation was greatest at site 3, the sandy silt loam, and least at site 1 (loamy sand), although there was considerable variation amongst replicates (Fig. 6).

c. Semi-automatic analysis.

Semi-automatic analysis used the same images as the fully-automatic analysis above. These were again processed in WinRhizo, but the threshold was set manually. The operator made a judgement for each image so that as much of the root length as possible was highlighted without including background material such as stones and light coloured soil particles as objects. As such it represents the best estimate of root length possible without the more time-consuming step of manually tracing roots.

Semi-automatic analysis resulted in estimates of root length much closer to those obtained by manual tracing than did fully-automatic analysis (Fig. 5). Nevertheless, there was some deviation and this varied both between sites and between replicate soil pits (Fig. 7). At site 1 (loamy sand), semi-automatic analysis consistently underestimated root length by up to 25%. At sites 2 and 3, on the other hand, semi-automatic analysis either underestimated (up to 52%), or overestimated by up to 97% (Fig. 7).

The type of lighting used in the photograph had a large effect on the visual quality of the image. Natural lighting revealed much greater detail of soil micro-structure and profile topography, but in doing so enhanced the variability of the background against which the roots were measured. Flashlighting reduced the variation in background, resulting in a more uniform and grey image, but it also reduced the contrast with the roots. Consequently automatic and semi-automatic analysis were unreliable even for flashlit photographs.

Two photographs were also analysed using software developed by Michigan State University (MSU) for the automatic analysis of images of roots captured from mini rhizotron tubes. It has been refined over many years and forms a core element of their internationally regarded Root Image Processing Laboratory. This software uses a selection system which takes into account the length:width ratio of objects in addition to their grey-scale intensity. Objects must have a length:width ratio greater than 5 to be accepted as a root. This software considerably underestimated root length compared to manual tracing using WinRhizo. Root lengths were only 22 and 27% of those determined by tracing (Table 3). Roots

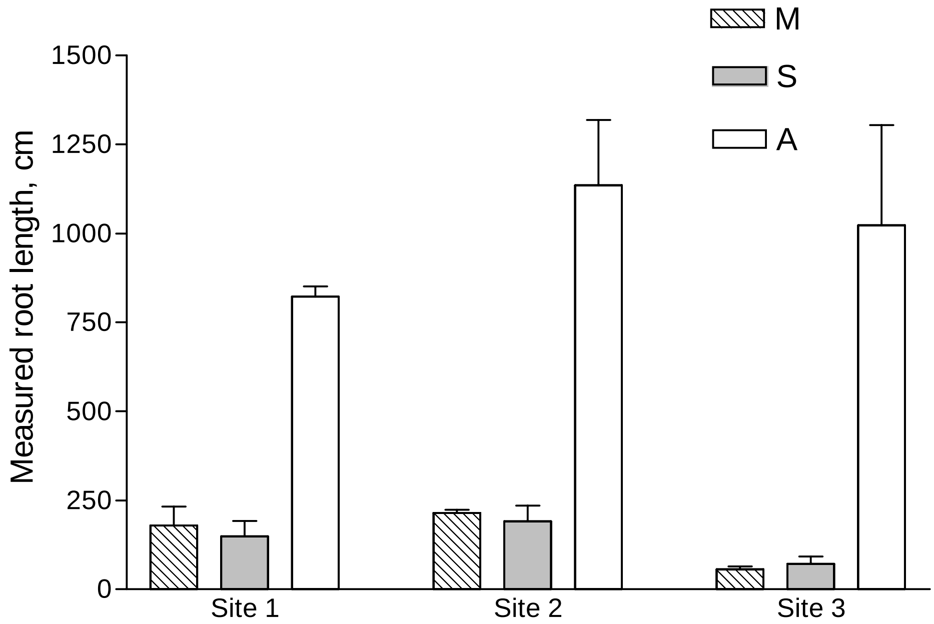

Fig. 5. Root lengths measured from images of the profile wall using manual tracing (M), semi-automatic (S) and fully-automatic (A) image analysis. Values are means ± SE of 3-4 replicate soil pits per site.

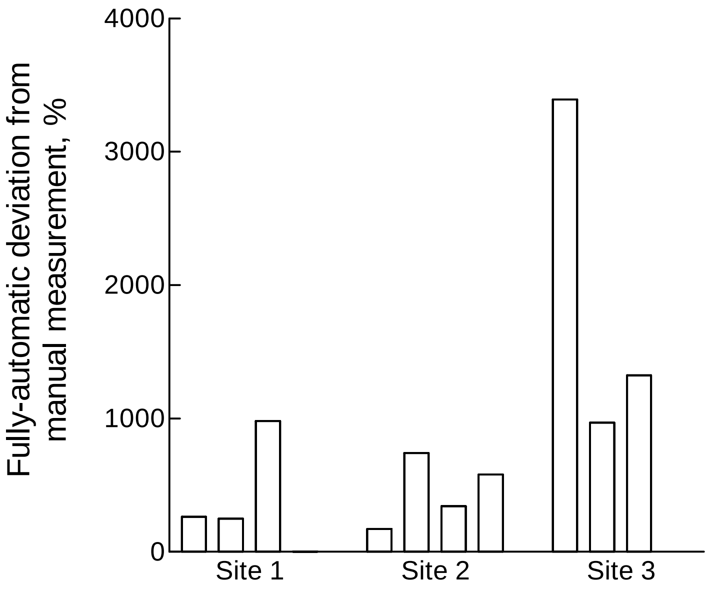

Fig. 6. Overestimation of root length by fully automatic analysis: variation between images of replicate soil pits

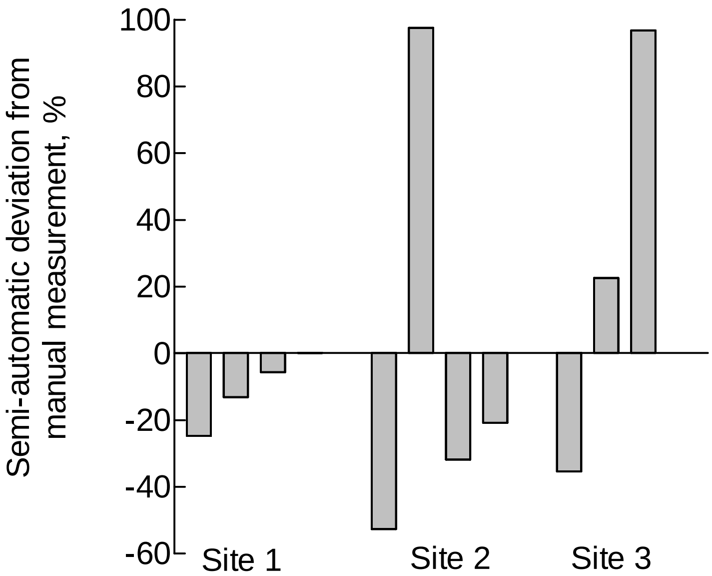

Fig. 7. Over- and underestimation of root length by semi-automatic analysis: variation between images of replicate soil pits

growing against a mini rhizotron tube are relatively clean and show good contrast against the background soil. The MSU software works well with these roots giving reliable and accurate measurements of root length. However, roots exposed on a profile wall are covered in soil and show relatively little contrast with their background.

Table 3. *Root length (cm) measured from two images using different image analysis techniques. WR refers to use of WinRhizo software for manual tracing, semi-automatic and fully-automatic (auto) measurement. MSU auto refers to automatic analysis using the Michigan State University software developed for minirhizotron images.*

Image	WR manual	WR auto	WR semi-auto	MSU auto
1	274	335	195	74
2	91	1574	77	20

To investigate the effects of image resolution on the measurements of root length, a comparison was made of lengths measured on photographs taken using a wide-angle and a telephoto (zoom) lens setting. The wide-angle photographs were of the whole 20 x 20 cm grid; a single 10 x 10 cm zone within the image was selected for measurement by manual tracing. The same 10 x 10 cm zone of the profile wall was also photographed using a telephoto setting, so that the particular zone filled the frame. Root lengths in this image were also traced manually. Four profile walls were selected to provide a range of root lengths. Fig. 8 shows the lengths measured from the zoom image plotted against those from the wide-angle image of the same profile and grid zone. There is good agreement between the two sets of measurements ($r^2 = 0.999$). The slope of the relationship is 1.06 (significantly greater than 1 at $p = 0.05$), which indicates that there is a small (6%), but significant increase in the length of roots measured from the zoom image compared to the wide-angle.

4.2 Root excavation

4.2.1 Influence of site and soil type

The target depth of air-knife excavation in experiment 1 was 5 cm. Excavation was relatively easy at sites 1 (loamy sand) and 2 (sandy loam), but more difficult in the sandy silt loam at site 3. At the latter, it took longer to remove the required soil and the soil tended to break off in larger aggregates. The

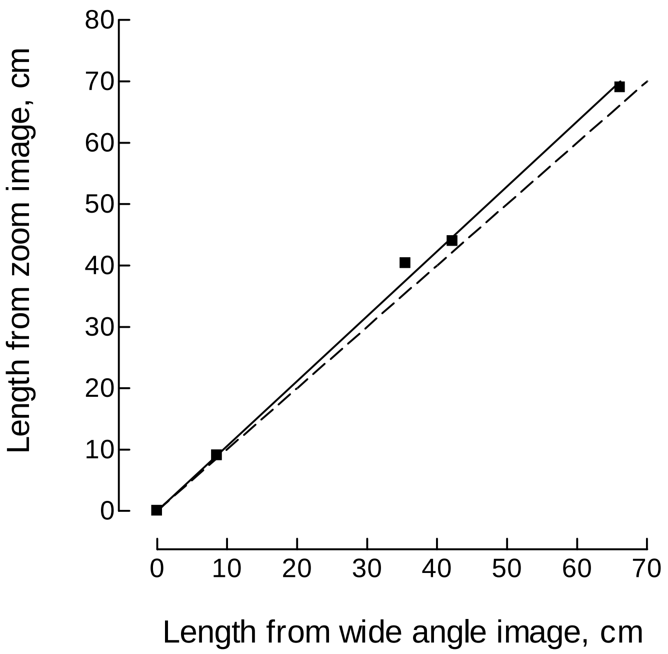

Fig. 8. Relationship between root length measured from close up (zoom) photographs of a particular zone on the profile wall and wide-angle photographs of the same zone. Solid line fitted by regression, $y = 1.06\ x$; $r^2 = 0.999$ (SE of slope = 0.0174). Broken line indicates 1:1 relationship.

uniformity of excavation that was achieved across the profile wall was assessed by examining the variation in depth between the nine points of the grid. Data for the three or four replicate soil pits at each site were pooled and are presented in the form of a column scatter plot (Fig. 9).

The absolute range of depths excavated between different points on the grid and between different soil pits was the same at sites 1 and 2 (i.e. 4-7 cm). The mean depth was also comparable; 5.3 and 5.2 cm for sites 1 and 2 respectively. However, the standard deviation for site 2 was larger and this can be seen in the wider spread of points across the range. These results indicate there was a greater variability in excavation depth across the profile wall and from pit to pit at site 2 compared to site 1. At site 3, excavation depth ranged from 3 to 7.5 cm, but the mean depth was 4.4 cm; short of the target of 5 cm. The large standard deviation at site 3 results from a relatively small number of points at the top end of the range.

These results are consistent with field experience of using the air-knife. After excavation, the profile wall was visibly more uniform at site 1, than sites 2 and 3. Soil at sites 2 and 3 had a greater stone content, and it was noted that at site 3 in particular, the soil appeared to have greater strength. This may have been due a combination of the relatively low water content for this soil texture (Table 4), and possibly a greater bulk density (not measured), rather than the differences in textural class. Whatever the cause, it was certainly more difficult to dig the soil pit and insert the theta-probe into the profile wall at site 3 than the other sites.

Table 4. *Volumetric soil moisture content of the profile wall in experiment 1. Values are means ± SE of 4 readings in each of 3-4 replicate soil pits.*

Site	Moisture content (% v/v)
1	10.0 ± 0.7
2	22.7 ±2.5
3	12.1 ± 1.6

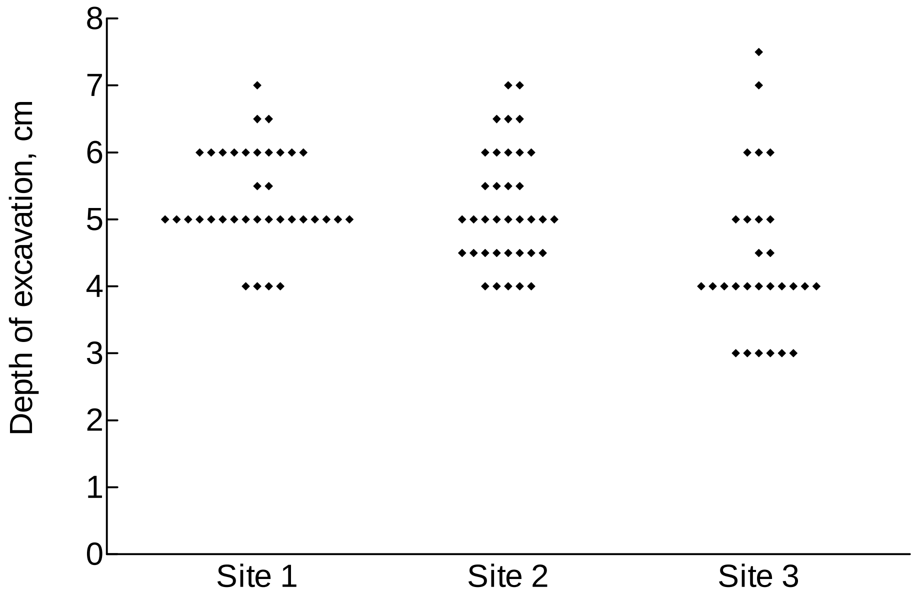

Fig. 9. Scatter diagram illustrating the extent of variation in excavation depth at each site. Each symbol represents the depth at one point in the 20 x 20 cm grid on the profile wall. Data for replicate profile walls have been pooled. Means ± SD for the sites were: site 1, 5.3 ± 0.7; site 2, 5.2 ± 0.9; site 3, 4.4 ± 1.2.

The air-knife works on the principle of forcing air into the soil air spaces at high pressure. As the jet of air is removed from an aggregate, thus removing external pressure, the high-pressure air inside the aggregate expands causing it to fragment. High soil strength would be expected to cause the soil to break up into larger sized aggregates along existing planes of weakness. Differences in soil strength between sites may, therefore, be one factor contributing to the differences in variation in the profile wall after excavation. In addition, loosening and removal of large stones would also lead to an irregular profile wall surface. Removal of one or two large stones or aggregates of soil would be enough to increase the range of excavation depths measured, but still resulting in a low average depth (as was found at site 3). The failure to reach the target depth of excavation at site 3 is a further reflection of the greater difficulty of excavation at this site.

4.2.2 Reliability of estimates of root length after air-knife excavation

Experiment 2 investigated whether air-knife excavation followed by image analysis of the exposed roots provides reliable estimates of root length. Soil was progressively excavated to a series of pre-determined depths, and at each depth photographs were taken.

As the depth of excavation increased, and a greater volume of soil was removed, there was no increase in the length of roots measured from images of the profile wall (Fig. 10). The apparent root length density can be estimated by first calculating the cumulative volume of soil removed at each depth of excavation. This is the product of the area of the grid (400 cm^2) and the mean depth of excavation. The root length density (cm cm^{-3}) is then given as root length/soil volume.

The mean (± SE) root length density, determined by soil coring was 2.25 cm cm^{-3} (± 0.21) for the soil layer 5-25 cm below the surface of the ground. Fig. 10 shows the apparent root length density after air-knife excavation, expressed as a percentage of that determined by soil coring. At the shallowest excavation depth, the apparent root length density was only about 40% of that estimated by coring. With increasing depth of excavation, the percentage root length density declined, reaching a value of only 7% at the deepest point. The almost constant root length measured with increasing depth of air-knife excavation is a consequence of the damage caused to the exposed roots. A large fraction of the root length is lost during excavation leaving a relatively fixed root length on the profile wall. If no root losses occurred, there should be a steady increase in root length measured from images of the profile wall, and a relatively constant root length density with depth of excavation.

However, when the profile wall was excavated to 0.5 cm by hand, using a trowel, the root length on the profile wall was 240 cm (Fig. 10). This represents a root length density of 53% compared to soil coring.

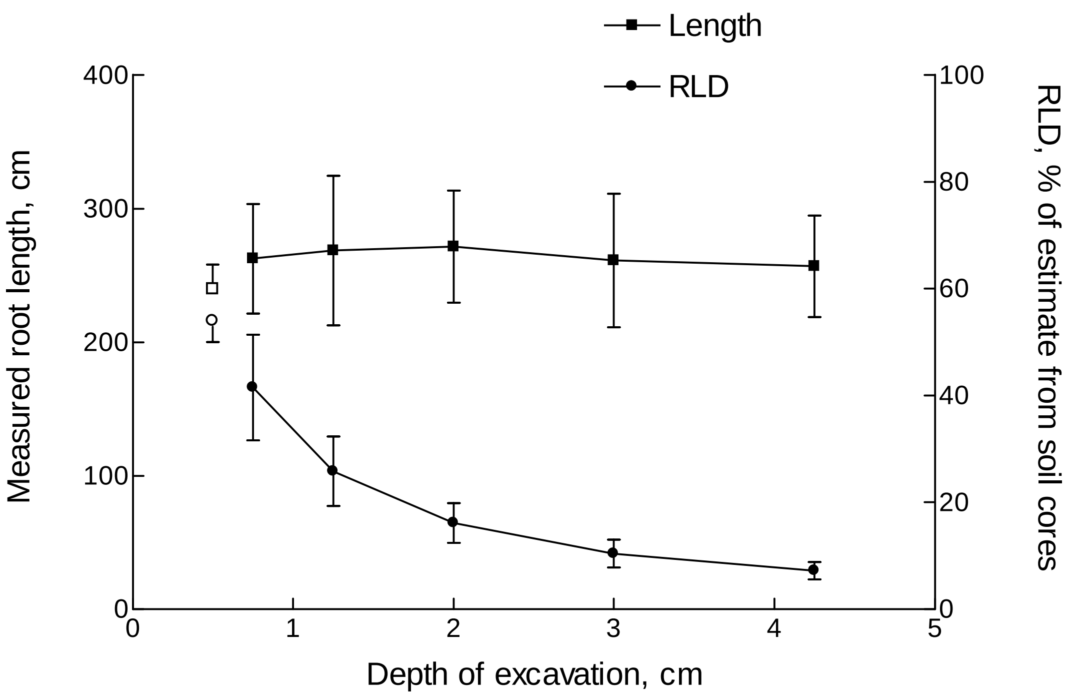

Fig. 10. Root length measured from images of the profile wall after excavating to different depths. Solid squares, root length after air-knifing; open square, root length after hand excavation; circles, estimated root length density (RLD; root length per unit volume of soil) as a percentage of that determined by soil coring (solid circles, soil excavated by air knife; open circle, soil excavated by hand). Values are means ± SE of 4 replicate soil pits (3 in the case of hand excavation).

The length was not significantly different ($p>0.05$) to that measured after air-knife excavation to an average depth of 0.75 cm.

4.2.3 Effects of mesh screening on root losses

An important factor contributing to root losses was thought to be movement of heavy soil particles, still attached to the end of roots, in the air-stream. The swinging motion may have caused the roots to break. In experiment 3, mesh screening was used in an attempt to reduce the movement of soil particles during excavation.

In a preliminary experiment, a range of mesh sizes was used to determine their effects on soil excavation. Fine mesh (1 and 2 mm gauge) was unsuitable as it impaired the process of excavation. Thus there was insufficient removal of soil when excavating through fine mesh. This is probably the result of a reduction in the velocity of the air-stream below the critical value required for penetration into the soil pore spaces. On the other hand, too large a mesh size would provide little benefit in reducing the movement of roots and soil particles. Thus a mesh size of 4 mm was selected for further evaluation. This was considered to be sufficient to allow some soil loosening by the air-knife, but small enough to restrict excessive movement of soil particles.

In the absence of a mesh screen, the mean length of roots recovered by excising from the profile wall was 1048 cm (Table 5). This represented only 30% of the total root length recovered, the remaining 70% was located in the excavated soil on the floor of the trench. With mesh screening, there was no significant increase ($p>0.05$) in the length of root recovered directly from the profile wall. Again, roots cut from the profile wall represented only 31% of the total length of roots measured. However, a great majority of the remaining root tissue (63%) was recovered from soil trapped behind the mesh; relatively little (6%) was found on the trench floor. This suggests that the screen was effective in reducing some soil movement, but not in preventing damage to the root system during excavation. The root length densities estimated from these excavations were approximately 2 to 3 times less than those derived from soil coring (Table 5).

Table 5. *Effects of air-knife excavation through mesh screening on recovery of roots from different locations. Values are mean root lengths (cm) ± SE for 4 replicate soil pits. Root length densities (RLD) estimated from soil trenches are based on mean values of the total root length recovered. Root length density from soil coring are means ± SE of 4 replicate groups of 8 (2.5 cm) or 4 (8.0 cm) cores taken adjacent to the soil pits.*

Location	With mesh	Without mesh
Profile wall	1433 ± 436	1048 ±317
In trench	307 ±106	2834 ±1070
Loose behind mesh	3021 ±942	-
Total	4760 ±1262	3882 ± 1119
Profile wall % total	31	30
Estimated RLD (cm cm^{-3})	3.97	3.24
RLD from soil cores		
2.5 cm diam	8.29 ± 2.05	
8.0 cm diam	9.90 ± 1.5	

5. DISCUSSION

There were two key elements to the project. The first was image capture in the field and its subsequent processing. The second was excavation of the soil using the air-knife.

5.1 Image capture and analysis

The results show that photographs of sufficient resolution for image analysis can be taken in the field using a good quality digital camera. Manual tracing of roots on the images was needed to measure their length. However, this was relatively quick and at the root length densities found in the top soil of a spring barley crop, tracing of roots in an area of profile wall measuring 20 x 20 cm took approximately 10 minutes.

Automatic and semi-automatic analyses of images were not reliable, even with two of the most sophisticated image processing software packages currently available (Fig. 5, Table 3). In contrast to automatic processing of mini rhizotron images (Andrén *et al*. 1996), it is unlikely that significant improvements in automatic processing of profile wall images will be made in the near future. The greatest difficulty lies in the fact that roots exposed at a profile wall are covered, to varying extents, with soil, reducing the contrast between the root and its background. In this case more elaborate segmentation routines based on colour would not help, because the colour of soil covering the root is much the same as that of the background. Washing exposed roots with water is unlikely to improve the situation much. In our experience, washing can lead to a smearing of soil over the roots, often reducing the contrast with the background compared to roots excavated by the air-knife. Wetting the soil also results in considerable reflection when using flashlighting, which enhances, rather than reduces, the problem of variable contrast during image analysis.

The image resolution in the wide-angle photographs (profile wall frame size 20 x 20 cm) was such that most of the roots had a diameter of two or more pixels. Although this is on the low side (ideally it should be 3 or more), it was adequate for manual tracing because grey-level thresholding was not used, and length rather than diameter was being measured. Increasing the image resolution four-fold (by photographing an area of profile wall 10 x 10 cm, rather than 20 x 20 cm) resulted in only a 6% increase in total root length measured (Fig. 8). This suggests that most of the roots present on the profile wall were visible in the wide-angle photograph. The small (6%) increase in accuracy would rarely justify the four-fold increase in work required to capture and process the higher resolution photographs.

Root lengths measured on profile walls after shallow excavation generally do not provide reliable estimates of root length density when compared to soil coring. Kücke *et al*. (1995) estimated root lengths of winter wheat and rye exposed on profile walls which had been excavated to a depth of 3 cm using a jet of water. Root length densities were 6 to 10 fold less than those obtained by soil coring, depending on soil type. Similarly, root length densities of maize estimated from profile walls excavated to a depth of 5 mm were approximately 50% of those determined by soil coring (Böhm 1976). The discrepancy between estimates made using profile walls and soil coring has been ascribed to losses of roots during profile excavation, or poor visibility of roots on the trench wall (Böhm 1976; Kücke *et al*. 1995).

In the present work, hand excavation of the profile wall to a depth of 5 mm and manual tracing of images gave a root length density 53% of that determined by soil coring (Fig. 10). Although some loss of root cannot be ruled out, excavation was done carefully and it is unlikely that losses can account for the scale of discrepancy observed. Nor is it likely that the discrepancy is due to a failure to see and measure fine roots. As discussed above, a four-fold increase in image resolution only gave an increase in measured root length of 6% (Fig. 8).

With the present system of measurement, an important source of error is likely to be the orientation of roots emerging from the profile wall. Whole roots, or sections of root, which are not lying parallel to the face of the profile wall will have their length underestimated. The error will be greatest with shallow excavations. In principle, a greater proportion of the length of non-vertically orientated roots will hang vertically after deep excavation than after shallow excavation, allowing more of their true length to be measured.

5.2 Soil excavation by air-knife

The second element of the project was to investigate whether the air-knife could be used for such ‘deep’ soil excavation. It is clear from the results that it cannot be used in the manner proposed for two reasons. Firstly, it is not possible to excavate the soil precisely enough to provide a reliable estimate of the volume of soil removed (Fig. 9). Secondly, and more importantly, it causes too much damage to the cereal root system. Consequently, the root length measured on photographs after excavation to a pre-determined depth is not a good estimate of the true root length density (Fig. 10). It was not possible to reduce the root losses by excavating through a mesh screen. Even though the movement of soil particles was reduced (as shown by the retention of soil behind the screen), there was no benefit in terms of the length of root remaining on the profile wall (Table 5).

5.3 Advantages of air-knife excavation and digital photography

Although excavation by air-knife, coupled to digital photography and image analysis, cannot be used for direct determination of root length density, the system does offer some advantages in standard profile wall assessments of root number and distribution. A protocol for such assessments, and estimates of the time taken to complete different stages of the measurement process, are outlined in Appendix A. If roots are traced during image processing to measure root length, the time required for one profile wall is very similar to that reported by Böhm (Böhm 1976; Böhm *et al*. 1977). However, the time required is considerably less if only root counts are made.

A major advantage of the technique is that data capture is more rapid. Most profile wall assessments involve direct counting of roots at the trench wall, or tracing of roots on to an acetate sheet for analysis in the laboratory (Böhm 1976; Böhm *et al*.1997, Kücke *et al*. 1995; van Noordwijk *et al*. 2000). Use of the digital camera can reduce the time spent in the field by up to 70% (Table 6 Appendix A). This allows the slower processing stage to be conducted in the more comfortable environment of the laboratory. Böhm (1976) reported a time requirement of 3 h, and van Noordwijk *et al*. (2000) 0.25 days (approximately 1.85 h) in the field per profile wall. This contrasts with only 47 minutes for the protocol in Appendix A. The speed of data capture in the field becomes important in climates such as the UK where prolonged periods of dry weather cannot be guaranteed. It also enables work to be organized more effectively, because once the images have been captured, analysis can be scheduled around other tasks.

There may also be potential time-savings in data analysis. Since the data are captured and stored in digital format, it could be more efficient than producing maps of root distribution by hand which then have to be digitized for analysis. However, estimates of the time required will vary greatly depending on the type and level of sophistication of the analysis being undertaken. Few studies provide sufficient detail of the work breakdown to permit accurate comparison. Van Noordwijk *et al*. (2000) have reported a time requirement of 1.5 days per profile wall for digitizing and analysing maps of root distribution.

The technique in Appendix A also offers advantages in terms of operator safety. In order to protect operators, deep trenches need to be shored up or wide enough to allow escape should the trench walls collapse. Using an air-knife with a long barrel to excavate the wall, and a digital camera lowered into the trench on a jig, all operations can be conducted from outwith the trench. This means that relatively narrow trenches can be used, which are quicker to dig and cause less disruption to the site. Only a relatively narrow range of soil types was studied in the current project. Excavation of clays using the air-knife is generally more difficult, because of their smaller pore size distribution. However, in the protocol outlined in Appendix A, shallow excavation is all that is needed to expose root ends. Thus there is no reason to suspect that the technique could not be used on fine textured soils, but further work would be needed to confirm this.

5.4 Soil coring or profile wall measurements?

Soil coring is the standard technique for determining root length density, but it is time-consuming and hence costly. In the present study, the average time required to take one soil core (30 cm long x 8 cm diameter), wash soil from the roots, remove debris, sub-sample and spread the roots for digitization, was 65 minutes. This is comparable to the 70 minutes required for soyabean roots in cores 30 cm long (recalculated from Böhm *et al*. 1977) and 91 minutes for maize roots in 30 cm x 10 cm diameter cores of silty clay loam (Kaspar, cited in Oliveira *et al*. 2000). When the full rooting depth is sampled, the time-input increases substantially. Böhm *et al*. (1977) reported a time requirement of 9.5 h to complete the analysis of one core to a depth of 180 cm. Moreover, several cores need to be taken to account for the spatial variation in root distribution. Coefficients of variation tend to be large (e.g. Kücke *et al*. 1995).

Profile wall methods, therefore, still have much to commend them. They are less time-consuming, can have lower coefficients of variation (e.g. Kücke *et al*. 1995), and provide visual information on root distribution and rooting depth that farmers can relate to. Although they do not provide reliable estimates of root length density without first being calibrated against soil cores (Kücke *et al*. 1995; van Noordwijk *et al*. 2000), they do provide quantitative measurements of root numbers and length at the profile wall. These may be useful for comparing the relative performance of different varieties or for investigating the effects of soil management practice on root growth. The choice of method, therefore, depends on the type of information required and the resources available. The air-knife and digital photography can help reduce the time and cost involved in profile wall measurements.

6. CONCLUSIONS

- Digital photographs taken of cereal roots on profile walls provide sufficient resolution for image analysis.

- The resolution was not affected significantly by soil type in the narrow range of soil types investigated.

- Image processing requires manual marking or tracing of roots prior to computer analysis of root length or number. Automatic or semi-automatic methods of analysis are unreliable, because of the variable nature of the background and its poor contrast with the roots.

- Excavation of soil from the profile wall using an air-knife leads to a considerable loss of roots.

- Measurements of root length exposed on the profile wall, either by hand or by air-knife, do not provide reliable estimates of root length density. This is largely due to errors associated with a loss of roots and the orientation of roots at the profile wall in relation to the plane of the photograph.

- A protocol has been developed for the quantitative assessment of root numbers on a profile wall using the air-knife and digital photography. The technique offers significant savings in time and cost over standard techniques. In particular, it is estimated that the time spent in the field can be reduced by up to 70%.

- The proposed technique also offers benefits in terms of improved operator safety.

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Appendix A

Outline protocol for profile wall assessment of root number and length

1. Dig trench with mechanical digger to expose a 1 m x 1 m profile wall.
2. Excavate the profile wall using an air-knife to expose roots.
3. Position a 1 x 1 m frame (divided into 10 x 10 cm grids) against the profile wall for alignment of the camera and calibration of photographs.
4. Photograph the profile wall in 20 x 20 cm frames using the camera attached to the jig and a remote shutter release.
5. Trace roots manually (or mark for root counts) on the digital images.

Estimated time requirement

In the present work, it took an average of 10 minutes to manually trace roots on photographs of a 20 x 20 cm area of the top soil, where the mean root length density (determined by soil coring) was 2.25 cm cm^{-3}. It took, on average, only 4 minutes to mark and count the roots on the same photographs. These times have been adjusted to account for the predicted reduction in root length density with depth (taken from Day *et al*. 1981), so that the total time needed to analyse photographs for an entire 1 m depth of soil can be estimated. A minimum time of 1.5 minutes was assumed for frames with no roots or roots at very low densities.

The estimated time for processing images of a 1 x 1 m profile wall is 120 minutes if roots are traced, or 60 minutes if roots are marked and counted (Table 6). This gives a total time for preparing and assessing a single profile wall of 167 and 107 minutes respectively.

In the protocol described above, the purpose of the air-knife excavation is to expose root ends so that they can be photographed, counted and their position mapped. Since root length density is not being determined, the depth of excavation is not critical. Also, the excavation depth was found to have little effect on the length of root retained on the profile wall (Fig. 9). This means that it is unnecessary to mark the wall and measure the depth of excavation. Consequently, all operations can be conducted from outside the trench with the benefit that only a narrow trench is needed. The required width will be dictated by the minimum distance necessary between camera and profile wall. A reference framework is needed against the wall, so that the camera can be aligned and the photographic images calibrated.

Data from Böhm *et al*. (1977) are presented for comparison (Table 6). These were derived from experiments on a crop of soyabean whose maximum root length density was only 43% of that of the spring barley crop used in the present experiments. The time estimates provided by Böhm *et al*. (1977) are based on trench profiles 180 cm deep. In order to compare the workloads involved in the above protocol with that of Böhm *et al*. (1977), data from the latter have been scaled to represent the same area of profile wall (1 x 1 m). The following assumptions have been made in the scaling process.

a) The time taken to excavate the trench, smooth the profile wall, wash the wall and refill the trench is a linear function of depth.
b) The time taken to estimate root length was the same as that published for the 180 cm deep trench, since there was little or no root below 90 cm (Böhm *et al*. 1977).

The total time required to prepare a profile wall and assess the roots was 171 minutes. This estimate (after scaling) is very similar to the 3 hours Böhm himself identified as the time needed to prepare and assess a 1 x 1 m profile wall in a maize crop using the same techniques (Böhm 1976).

Table 6. *Estimated time (minutes) required for different stages in the preparation and assessment of root length or number on a 1 x 1 m profile wall. Estimates based on measurements made in the present study and by Böhm et al. (1977)*.*

Present study		**Böhm et al.**	
Dig trench (mechanical digger)	10	Dig trench (mechanical digger)	17
Excavate with air-knife	5	Smooth profile wall by hand	50
Photograph roots in 20 x 20 cm frames	25	Wash and excavate soil from profile wall	33
Image analysis - root length - root number	 120 60	Estimate root length at wall	60
Refill trench	7	Refill trench	11
Total - **with root length** - **with root number**	 **167** **101**	**Total**	**171**
Total time in field	**47**	**Total time in field**	**171**

** data from Böhm et al. (1977) have been scaled to represent a 1 x 1 m profile wall*

Appendix B

Glossary of selected terms used in image analysis

Dots per inch (dpi). The number of "dots" or discrete elements (pixels) contained per inch of image processed. It is the most commonly used measure of resolution.

Grey-scale. Often referred to as "black and white" images, grey-scale images are in fact made up of discrete shades of grey (the exception to this is a 2-bit image, which contains only black or white). The grey-scale images used in the present study were 8-bit and thus had a possible 256 discrete levels of grey between pure black (0) and pure white (255). Higher-bit grey-scale images contain more shades of grey. But for the image analysis requirements of this project 256 was sufficient.

JPEG. Abbreviation for Joint Photographic Experts Group. JPEG is an internationally standardized file format for compressing data representing still images. The JPEG format results in some loss from the image. In other words, the decompressed image is not identical to the original one. JPEG is designed to exploit known limitations of the human eye, notably the fact that small colour changes are perceived less accurately than small changes in brightness i.e. compression is achieved by prioritising adjustments in colour over brightness. Thus, JPEG is intended for compressing images that will be looked at by humans. This is a potential problem if the images are intended for computerised analysis. A useful property of JPEG, however, is that the amount of information lost can be varied by adjusting compression parameters. In this study we used the least compression possible, producing files that were large, but loosing a minimal amount of information.

Pixel. Is the smallest element of an image that can be assigned a grey-scale value. The pixel size in the images used in this study depended on the dpi set at the time of scanning of washed roots or digital photography of profile walls.

Resolution. A measure of the ability of an optical system to form separate and distinct images of two objects that are close together. In this study it was selected by setting a dpi for the imaging operation. High resolutions (e.g. 600 dpi, giving a pixel size of 0.042mm) allow for more precise measurements, but produce very large file sizes and are slow to process. Thus it is desirable to select the minimum pixel size that produces reliable results. At a given camera setting, the resolution also depends on the area being photographed. For example, photographs of a large area of profile wall will have a lower resolution than a close-up photograph of a smaller area.

Threshold. The value of grey-scale intensity used to separate pixels belonging to the root from those that belong to the background. Thus pixels with a value higher than the threshold are considered as root and included in the analysis, whilst those with a lower value are discarded.

TIFF. Tagged-Image File Format. The TIFF file consists of a number of labels (tags) which describe certain properties of the file (such as grey levels, colour table, byte format, compression size etc). After these initial tags comes the data. The TIFF format is accepted and supported by most image processing tools for both workstations and personal computers. The TIFF format supports various compression routines (e.g. LZW), but the images used for analysis by WinRhizo in this study were all uncompressed.