

## A new dimension to observations in minirhizotrons: A stereoscopic view on root photographs

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**Summary** With a stereoscope, as used for the inspection of aerial photographs, sequential photographs of roots obtained by the endoscope method from 'minirhizotrons' can yield much more information than hitherto. A series of photographs shows that most of the roots seen in a minirhizotron in grassland grew on the surface of the lexan tube, while there was a gap between the roots and the soil. Decay of the extensive root hair zones around the roots may make new root growth in the gap between rhizotron wall and soil invisible. Some consequences of these observations for the endoscope method are discussed.

### Introduction

Since Sanders and Brown<sup>9</sup> published an improved method for root observation through tubes, 'minirhizotrons', in the field using fibre optics for photography, many root researchers have added small modifications to the method<sup>3,5,12</sup>. The advantages of the method are clear, as it is a nondestructive method, allowing frequent root observations in more replication and under more natural conditions than the large rhizotrons<sup>7</sup>. When compared with root densities obtained by washing soil samples, the relative amount of roots seen on the photographs were found to vary with depth in many cases.

Since 1980 we have used the method and performed calibration measurements on washed soil samples, for various crops and soil types. As improvements to the method we inserted insulation material into the tubes to prevent water condensation on the rhizotron wall and developed a technique for carefully inserting the tubes into prepared holes, pushing the tube upwards by placing plastic bars on the lower side to improve contact between soil and minirhizotron wall. Various angles for placing the tubes and various positions relative to crop spacing were tested. A 30-degree angle to the horizontal appears to be a reasonable choice for agricultural applications.

Still, some of the results are poor, as shown for example in Figure 1 for a grassland experiment. In the upper 10–15 cm of soil, root density on the photographs is relatively low, while the highest root density is observed at about 20 cm, which is not in agreement with washed samples. Initially we thought that disturbance of the local water balance might be responsible, as capillary rise to the soil above the tube is impeded. In early spring and during wet conditions, however, the phenomenon can be seen as well. In a number of crop/soil combinations problems occurred caused by a poor visibility of the roots, especially older brown roots. The use of colour film gave some improvement, but still the pictures were rather vague in certain periods (especially for sugar beet and grass on loam soils). The possibility of clay adhering to the tubes was considered, so the tubes were extracted for cleaning and then re-inserted. This procedure may damage the roots and gives only a temporary improvement. It remained uncertain whether the phenomenon is the result of the observation technique or a consequence of the observation situation of roots growing along an unnatural interface.

For the interpretation of aerial photographs it is common practice to use a stereoscope on sequences of partially overlapping photographs<sup>4</sup>. The stereoscope makes it possible to see a three-dimensional image. We decided to test this method on root photographs. A similar technique has been used to analyze X-ray radiographs of soils containing earthworm channels<sup>8</sup>.

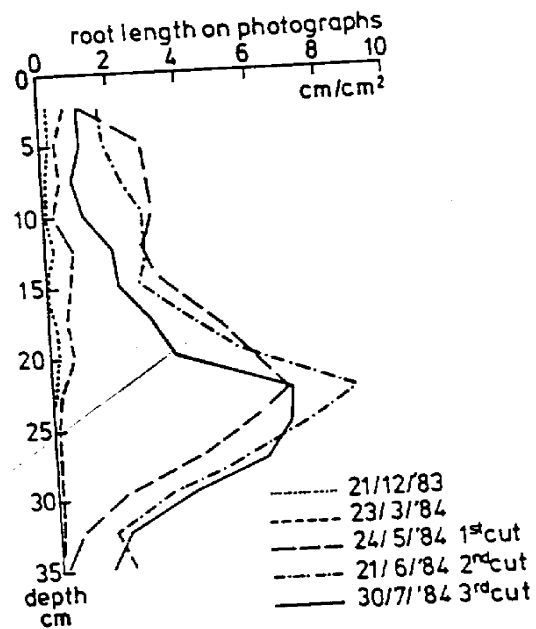


Fig. 1. Root length measurements on photographs in a one-year old grassland experiment.

#### Methods

Most of the details of the method for root photography as developed at our Institute have been described by Vos and Groenwold<sup>12</sup>. As minirhizotrons we use square tubes of 8 x 8 cm outside measurements made by welding 4 mm lexan (a polycarbonate which is more scratch resistant than the polyacrylic plexiglass) sides on a hardwood mold of exactly the right size.

A technique was developed for inserting square tubes into prepared holes in undisturbed soil profiles. A support is fixed in the topsoil and guides an auger at the desired angle with the horizontal. With the auger a cylindrical hole is made to the required depth. Subsequently, this hole is extended by hammering (or pushing hydraulically) a square sharp-edged metal tube slightly larger (5 mm) than the external measurement of the minirhizotron tube into the hole. After the tube has been placed, a v-shaped plastic bar (4 mm thick) is pushed into the soil to increase the contact of the tube with the soil. A block of polyurethane foam is placed into the tube as insulation material and removed only when photographs are taken.

For the present purpose we used a series of overlapping photographs (of a 6 cm diameter circle on the rhizotron wall) taken every 2.5 cm of the rhizotron wall. The rhizotron (lexan tube of 8 x 8 x 130 cm) was placed at an angle of 30 degrees to the horizontal in 1-year old grassland on a sandy soil. Photographs were taken in August 1984, after a dry period of one month.

For the observations a simple stereoscope was used (manufactured by Casella, London). Figure 2 shows how the stereoscope can be used to give a three-dimensional view on the zone of overlap between two photographs.

#### Results and discussion

Figures 3-6 show photographs at various depths. The reader has to use a stereoscope to see the three-dimensional effect.

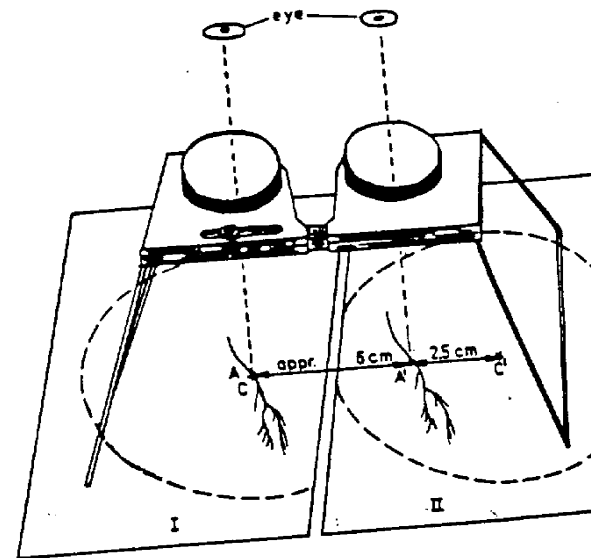
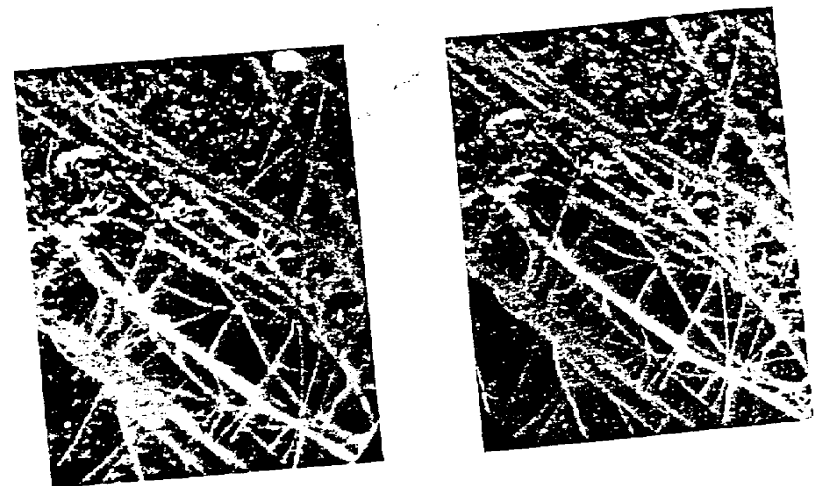


Fig. 2. Schematic view of the use of a stereoscope for observing overlapping photographs (I and II). Root A which is seen in the centre C of photo I is seen as A' in photo II, 2.5 cm away from C', the centre of photo II. For stereoscopic vision the distance AA' should be circa 6 cm.



Figs. 3-6. Root photographs mounted for inspection with a simple stereoscope. Fig. 3. In the upper part some older roots are seen between sand grains, in the lower part a distinct gap exists between the lexan tube and the soil. Most roots are growing tightly adherent to the tube, with extensive root hairs on both sides; some roots are growing in the open space (photographs at about 26 cm depth).



Fig. 4. A mat of older, disintegrating roots and root hairs is seen adhering to the tube wall. A young white root is growing behind this mat, in the gap between tube wall and soil (at about 24 cm depth).



Fig. 5. A greyish 'mist' has been formed on the tube wall, with young white roots just visible behind it (at about 18 cm depth).

The stereoscope opens up a new world of interpretation of root photographs and forms an important addition to the method, comparable to the use of a binocular microscope in large rhizotrons<sup>1</sup>. From the preliminary observations reported here some tentative conclusions can be drawn. The gap between the lexan tube and the soil is considerable (several times the diameter of a root) in the majority of the photographs. Most of the roots grow directly along the lexan tube, forming abundant root hairs. Judging from the angle most roots make with the base of the photographs, the conclusion can be drawn that roots do not follow the tube preferentially but rather proceed in their original direction as much as possible. By contrast, in subsoil, potato roots were seen to follow the tube (the preferential angle of the roots on the

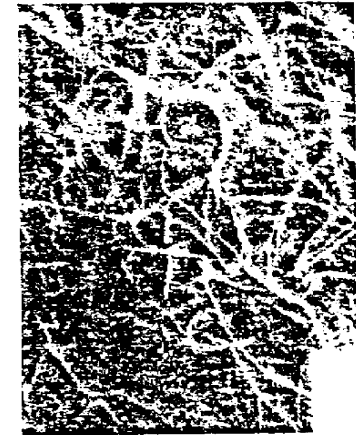


Fig. 6. Disintegration of old roots has proceeded far enough for new young roots to be visible (at about 4 cm depth).

photographs provides an easy check). In the figures shown, some branch roots penetrate the soil from a main axis along the rhizotron wall. Apparently the roots grow in quite an unnatural situation, although in soils gaps of comparable magnitude may exist as one of the 'microsites' for root growth. Figure 7 shows roots growing along aggregates in a clay soil which may be comparable to a situation in which the roots grow along the tubes. Some theoretical implications of soil root contact in such cases have recently been described<sup>7,13</sup>.

Decay of roots, root hairs and root exudates may result in 'debris' which obstructs the view on new roots growing behind it. This fact may cause large errors in estimating root length on the photographs. The stereoscope may help to see the roots in some cases, but still many roots will be overlooked. For a quantitative assessment of root length the endoscope method will therefore be unreliable. Keeping the tubes for a long time in permanent grassland would seem to have the advantage of improving soil/tube contact due to soil settling along the tubes, but apparently roots may become largely invisible. This effect is strongest in zones which previously had a high root density. Regularly removing the tubes for cleaning may be a way of overcoming these problems, but this carries the risk of damaging the roots. By first extracting the v-shaped plastic bar from the lower side of the tube the risk of root damage may be reduced.

In the literature reference is made to an influence on root growth of the rhizotron material. Voorhees<sup>11</sup> observed root growth behind plexiglass to be much poorer than in bulk soil. Electrical charges on the wall and water-repellency are possible explanations. Taylor and Bohm<sup>10</sup> found more roots behind acrylic plastic than behind glass; they attributed this fact to a better contact of the glass wall with the soil. The use of lexan, which we selected because of its strength and hardness, has not been previously described in this context.

The endoscope method may still give an opportunity to study the dynamics of root development, root branching, root longevity and root decay, especially as root hairs can be studied as well. In any situation one has to consider, however, whether or not the root may experience conditions different from those in the soil that favour local branch root development on the rhizotron wall. As regards root longevity, the rates of decay of roots and consumption of roots by soil fauna may be larger than in bulk soil. In a series of photographs of a potato crop we found two instances of a root which had disappeared (with all its branch roots) in a two-week interval, probably consumed by one of the larger members of the soil fauna. In one of these cases the roots had grown in an earthworm channel which had been

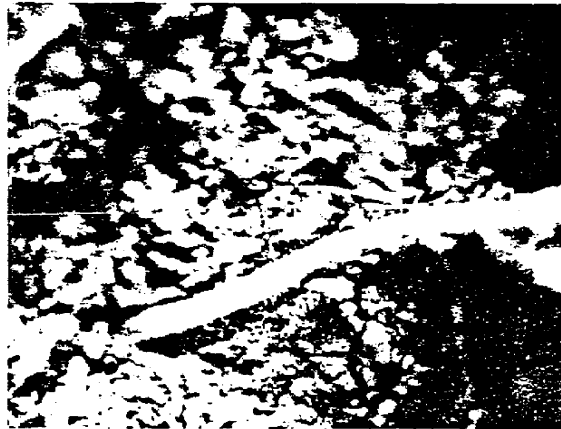
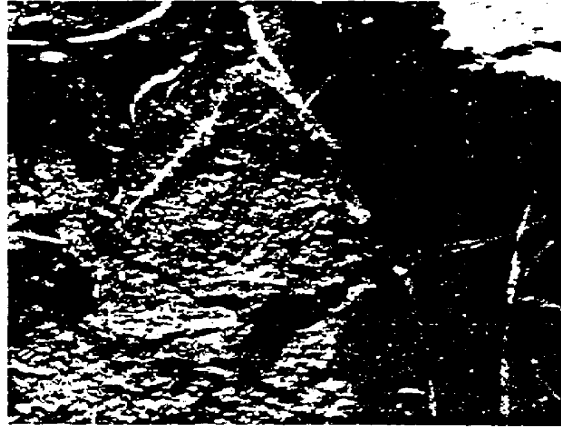


Fig. 7. Soil/root contact in a clay soil, showing partly immersed roots on aggregate walls (A) and roots growing freely in larger soil pores having soil contact through its root hairs only (B).

accidentally formed along the wall of the tube. Gaps and channels may be easily invaded by roots, but may carry increased risks of root damage as well. The same may be true for the smaller gaps between the rhizotron wall and the soil.

The endoscope method appears to be best suited to provide information on root growth dynamics. Destructive root sampling requires such high numbers of replicates at any time\*, that it is virtually impossible to obtain detailed information on changes throughout the growing season. Detailed observations on root photographs are useful for this purpose when they are combined with data from destructive root sampling techniques for calibration purposes. The stereoscope will add important information in such a procedure.

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