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An inflatable minirhizotron system for root observations with improved soil/tube contact

A.J. GIJSMAN, J. FLORIS, M. VAN NOORDWIJK and G. BROUWER
Institute for Soil Fertility Research, P.O. Box 30003, 9750 RA Haren, The Netherlands

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Abstract

Commonly used minirhizotrons consisting of a transparent tube inserted into the soil seldom attain good contact between the tube and the soil, which leads to root growth occurring in a gap rather than in the soil. A new system is described involving an inflatable flexible rubber wall, made from a modified motorcycle tube. Pressure ensures a proper tube/soil contact so that the environmental circumstances for root growth along the tube more closely correspond to those in the undisturbed soil. Before the endoscope slide is introduced into the minirhizotron for taking pictures, the inflatable tube is removed, so that there is no – often opaque – wall between the endoscope and the roots. This improves the picture quality and facilitates the analysis of root images.

Introduction

Minirhizotron systems are widely used as a method for determining root distribution and dynamics of root growth and decay in the soil (Brown and Upchurch, 1987; McMichael and Taylor, 1987; Van Noordwijk, 1987). Since the first reports on the method, using glass or acrylic plastic tubes inserted into the soil, several changes have been proposed and procedures for analysis of the root image data have been developed. However, one of the main problems of the minirhizotron system, i.e. how to obtain a good contact between soil and tube, has not yet been solved satisfactorily.

The reliability of the results obtained with a minirhizotron largely depends on whether or not root growth and decay observed along the minirhizotron wall corresponds to the actual root dynamics in undisturbed soil. In principle, the minirhizotron system should satisfy the following requirements:

* the tube wall material does not interfere with root growth or decay;
* a good soil/tube contact exists without compaction of the interfacial soil layer;
* soil temperature and soil water content should be the same in the bulk soil and the plane of observation;
* observing the roots should not affect root growth or decay;
* the visibility of the roots through the minirhizotron wall permits analysis of root images.

Several authors reported that poor soil/tube contact may be a major problem in minirhizotron measurements, as it not only results in aberrant root growth along the tube, but also in a reduced visibility of the roots growing in the gap between the minirhizotron wall and the soil. Upchurch and Ritchie (1983) showed that the existence of these gaps can lead to the formation of bunches of roots or to tracking of roots along the surface of the tube. In soils with dense layers impeding root penetration, a tunneling effect can be created by these gaps resulting in deeper rooting along the tube than in the undisturbed soil (Vos and Groenwold, 1987). Taylor and Böhm (1976) found that roots proliferated more strongly when they grew along the acrylic plastic rhizotron wall than within the bulk soil, which they attributed to a poor contact between wall and soil. In the topsoil, the existence of gaps around the tube may allow light to enter the root environment. Levan et al. (1987) found an 80% reduction in root length density of soybean roots growing in the upper 10 cm of the soil along a tube with deliberately created light leaks.

In an earlier publication we reported using a stereoscope for interpretation of root photographs (Van Noordwijk et al., 1985). By this technique a three-dimensional image of partly overlapping photographs can be created showing whether roots are growing along the minirhizotron wall or in a gap behind it. In most photographs distinct gaps of sizes several times the root diameter could be seen. In some cases the presence of gaps can be inferred from condensation of water on the tube wall, interfering with visibility of the roots (Van Noordwijk et al., 1985). Insertion of insulation material into the tubes reduces temperature differences and, hence, water condensation; this improves visibility, but the gaps as such remain. As there will always be small or large irregularities in the soil surface, originating from soil cracks or due to the insertion of the tubes, a good soil/tube contact can only be obtained when a tube with flexible walls is pressed against the soil. Recently, several systems have been proposed. Box and Johnson (1987) used polycarbonate tubing with an outer diameter slightly larger than the hole. By pushing the tube into the hole with a push rod the tube is stretched slightly so that its diameter becomes smaller; the tube can then be introduced into the hole without damaging the soil wall. After the force of the push rod is released the tube expands against the soil.

Inflatable tube systems were proposed by Maertens (1987) and Merrill et al. (1987). The first author describes a tube with a flexible outer membrane, which is transparent and can be inflated so that it presses against the soil. Details of the system have not been published yet. Merril et al. (1987) used a system consisting of a rigid inner cylinder acting as a frame, with a sheeting of transparent vinyl fixed upon it, which can be inflated so that it presses against the soil. A comparable system based on the same principle of a pressurizable wall was already used by Merril and Rawlins (1979), working with ports covered with flexible sheeting positioned in a lysimeter wall. None of these systems, however, seems to be completely satisfactory, because repeated air leakage from the pressurized air section occurs, and the problem of opaqueness of the wall is not solved. If rooting is not representative along the tube, or the recording of the root growth data is unreliable, the minirhizotron system can only give results of limited usefulness. Or as Smucker et al. (1987), working with image analysis of video-recorded plant root systems, wrote: 'No image-analysis procedures are capable of producing reliable root quantification information, if there is a fundamental problem with the rhizotron soil/tube interface'.

The minirhizotron system presented here is also based on the idea of an inflatable wall, but uses a very simple system, cheap, easy to use and guaranteed air-tight: a motorcycle tube. Experiences with a large number of lexane-walled minirhizotrons and inflatable minirhizotrons are discussed. Special attention is given to root visibility and to the position of the roots in the soil/tube interfacial area. Results of a small experiment to test the effect of different air pressures in the tube are presented as well.

Materials and methods

The first minirhizotron system we used consisted of 8 × 8 cm lexan tubes inserted into the soil, as described by Van Noordwijk et al. (1985). Photographs were taken with an endoscope mounted in a sliding support which fitted tightly into the observation tubes (Vos and Groenwold, 1983). This system, having rigid walls, possesses the same drawbacks as described in the introduction. We therefore replaced the lexan tubes by a metal frame (aluminium or stainless steel) consisting of 1-cm-wide ribs, with the remaining 6 cm between the ribs left open (Fig. 1). An inflatable rubber tube, made of a cut motorcycle tube and stretched by a metal rod, was intro-

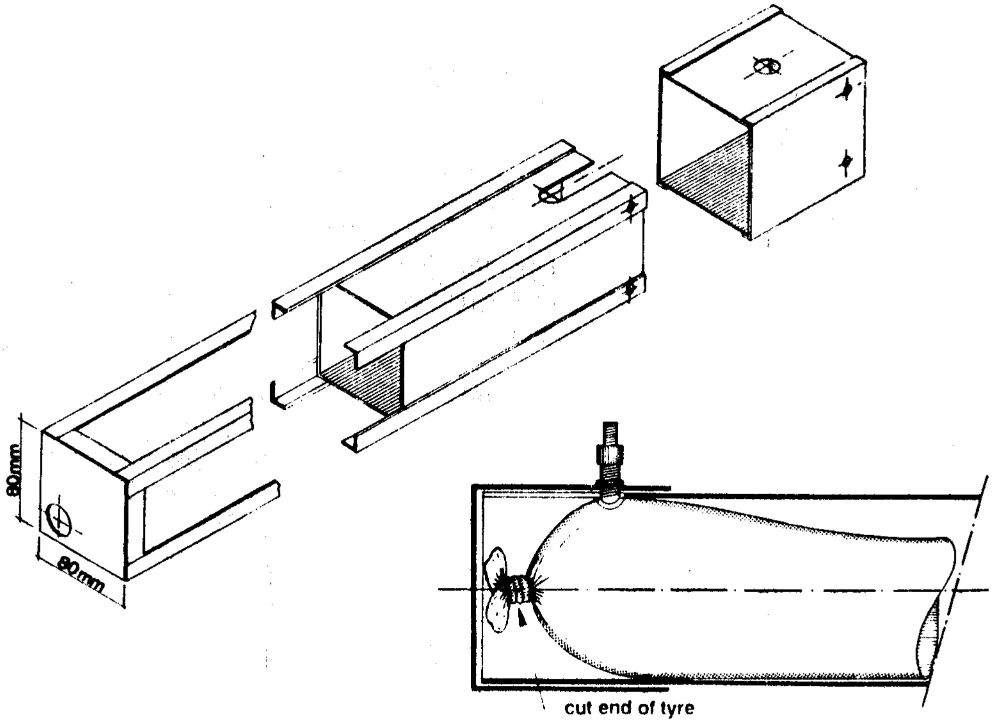

Fig. 1. The metal frame of the minirhizotron and the connection between the tube and the cap of the frame.

duced into the frame and pressurized. The tube bulges between the ribs (Figs. 1 and 2) and presses against the soil. The valve of the tube fits into a metal lid covering the frame and protruding above the soil. The metal rod that stretches the tube during positioning into the frame ensures that it cannot crease while being inflated. A low pressure of 0.12–0.13 MPa (thus an over-

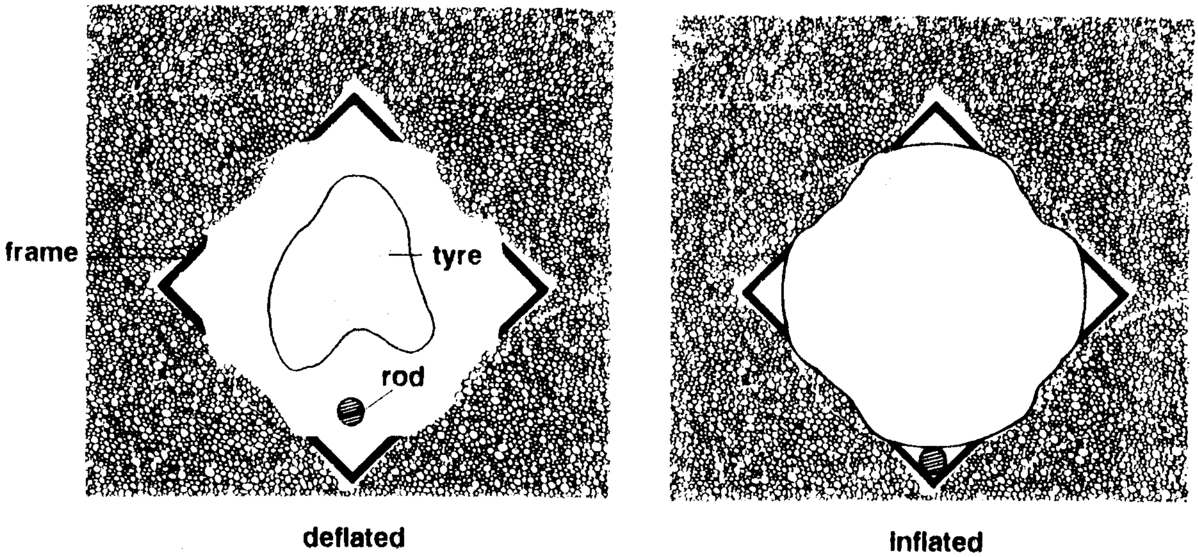

Fig. 2. Cross section of the minirhizotron system installed in the soil, with inflated or deflated tube.

pressure of 0.02–0.03 MPa compared with the atmospheric pressure), was sufficient to press the tube tightly against the soil. A higher pressure probably would result in compaction of the interfacial soil layer.

When photographs have to be taken, the tube is deflated and removed from the frame (Fig. 2). This has to be done very carefully so that the roots continue to adhere to the soil and do not change position. Now the sliding support containing the endoscope and the camera is positioned in the hole, guided downwards by the ribs of the metal frame. At regular intervals a photograph is taken of the roots without any glass/acrylic wall or vinyl sheeting between the endoscope prism and the roots. The visibility of the roots is therefore not limited by opaqueness of the minirhizotron wall. After the photographs have been taken, the sliding support is removed and the tube is brought into position again and inflated. This procedure can be repeated several times during a growing season with little disturbance of the roots.

An important point is the type of motorcycle tube, to be used in the frames. It should be supple enough to follow the irregular shape of the soil surface in the hole; at the same time it should not be too thin in view of the risk of leaks caused by sharp stones or pieces of wood in the soil. Most high-quality tubes were found to be too tough, but a Taiwanese make (HWA FONG size 2.75/3.00-21) was highly satisfactory. A brick should be placed on the spot where the minirhizotron touches the soil surface, because the thin layer of soil overlying it may be pushed up by the bulging tube. When working with grass this is not necessary as the dense mat of roots and stolons prevents the upward movement of the soil.

Originally we used this type of minirhizotron with aluminium frames in agricultural soils of neutral pH, where no danger of corrosion of the aluminium or deterioration of the rubber existed. Recently, however, the system was used in a sandy forest soil with pH values varying between 3.2 and 4.0 at different depths, which may be sufficiently acid to corrode the aluminium, although it was of seawater-resistant quality. We therefore switched to stainless steel frames. As the rubber tube contains large amounts of zinc that could be released at low pH values, we wrapped the tube in a polyethylene envelope. Both the tube and the envelope were removed when photographs were made and were reinserted afterwards.

Over the years, a large number of observation series were made for a variety of crops and soils. For the present comparison, representative photographs of each series were evaluated for root visibility, position of the roots in the soil/tube interfacial area, abundance of root hairs, and formation of condensation. To investigate the effects of tube pressure, an experiment was carried out with inflatable minirhizotrons installed in permanent grassland on a sandy soil and on a clay loam soil, using different tube pressures. Starting in autumn 1989, photographs were taken at four different times during the following winter and spring.

Results and discussion

The inflatable minirhizotron system was used for several years with sugar beet, wheat, grass, Douglas fir and apple tree, both in sandy and heavy clay soils. The photographs taken with the new system (Fig. 3) are much clearer than those taken in the lexan tubes. No condensation, smears or growth of algae obscure the visibility of the roots. Repeated observations during the year offer a good opportunity to follow root growth dynamics during the season: Figures 3C and 3D show how roots of sugar beet were fully embedded in the soil when they were young and fully turgid (date 21 July), but lay loosely in the earlier created channel at a later stage (date 5 October), when their diameter had decreased due to disintegration of epidermis and cortex.

In Table 1 a comparison is made of results on root visibility and root performance in both types of minirhizotron systems. Although in only one year a direct comparison of the two methods was made, the results for 17 series (different years or locations) with lexane tubes and 12 series with inflatable tubes show important differences between the two methods. In the lexane-walled system, root visibility was often poor, due to smears of mucigel or decaying root hairs, and soil particles settling on the lexane wall resulting

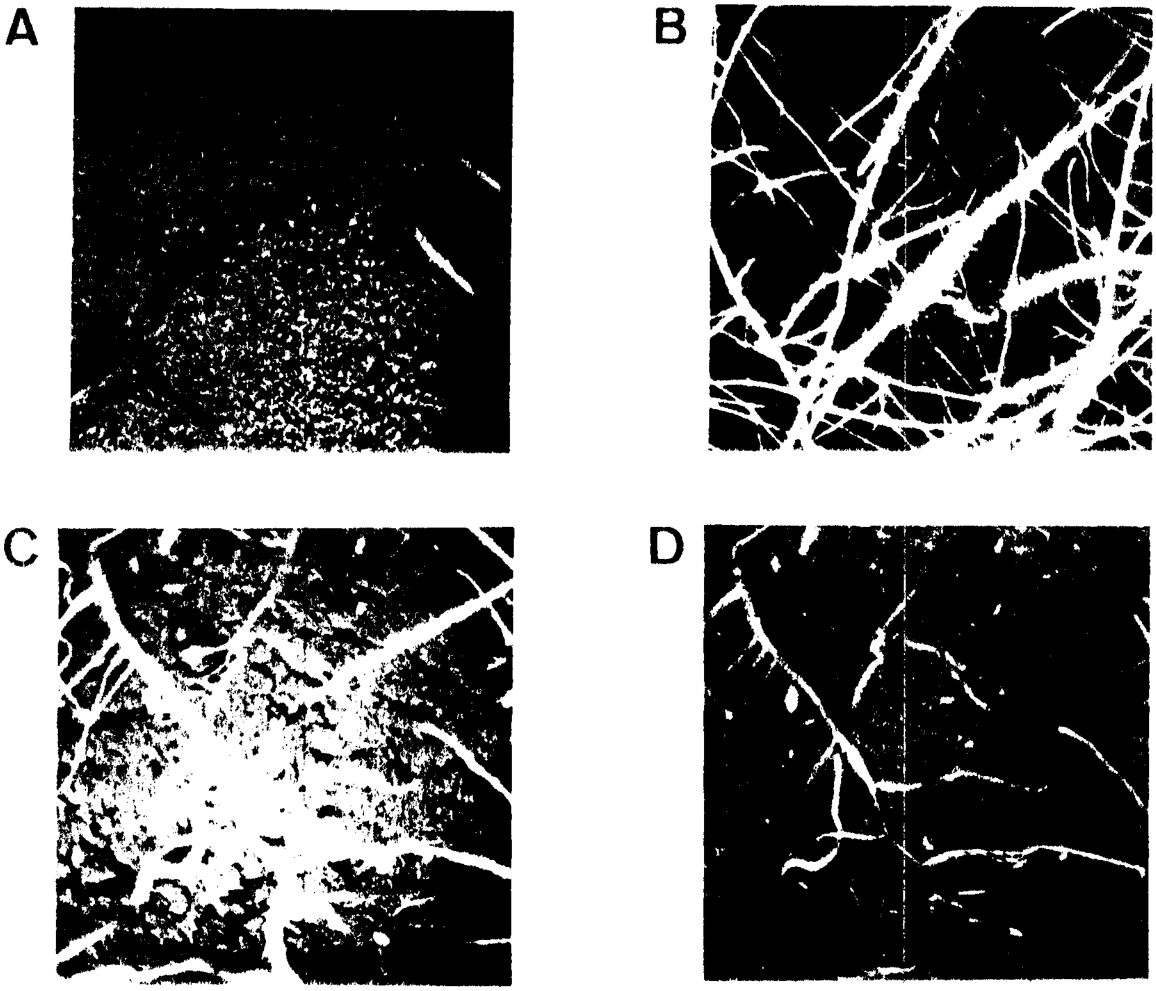

Fig. 3. Root photographs taken with the new minirhizotron system. **A**. Douglas fir on sandy soil. **B**. grass on sandy soil. **C** and **D** sugar beet on a heavy clay soil, at different times of the year at the same location (21 June 1987 and 5 October 1987, respectively).

from internal slaking of the soil or condensation of water; condensation could be largely avoided by inserting insulating material into the lexane tubes. Use of a stereoscope on partly overlapping photographs revealed that roots mostly grew along the lexane wall (Fig. 4A) or in the gap between the wall and the soil surface (Fig. 4B). Also, the abundance of root hairs may indicate that soil-root contact was poor. With the inflatable minirhizotron system, root visibility was always very good. Roots now grew along the soil surface (Fig. 4C) or were embedded in the soil (Fig. 4D); root hairs were only occasionally observed, as the roots were in direct contact with the soil. Initially we expected that roots might adhere to the tube via root hairs, but this was not the case.

Sometimes it was observed that roots grew along the frame ribs instead of following their own growth direction, or grew over the edge of the ribs. This could mostly be attributed to ribs being too thick or to the tube pressure being too low, resulting in the formation of voids between the tube and the soil surface. By beveling the sides of the ribs the transition from rib to soil can be made more gradual, so that gaps can no longer be formed. Also, when roots have only very short root hairs, or none at all, and therefore do not strongly adhere to the soil, or when roots are rigid and tough (as with trees), there is

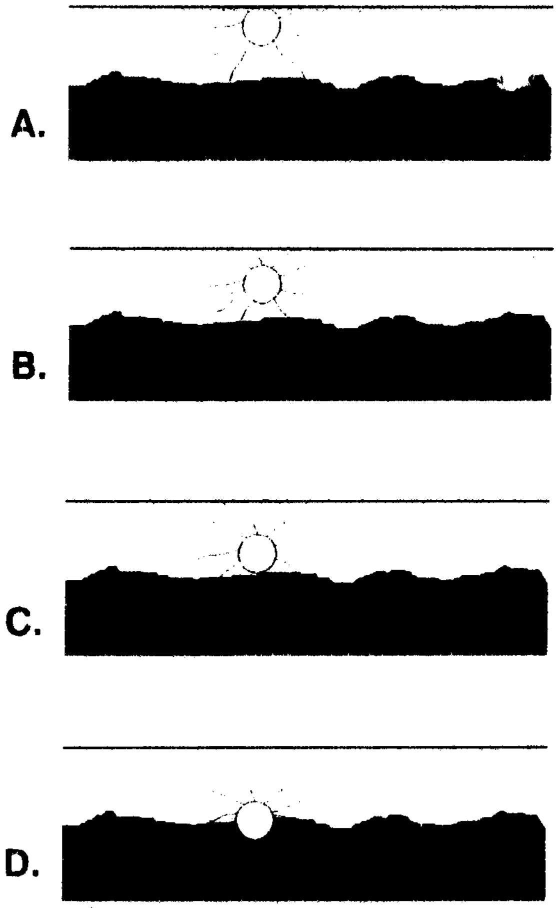

Fig. 4. Possible positions of roots with respect to soil surface and minirhizotron wall (rigid or flexible).

a chance that roots are shifted or that root tips hang freely in the frame interstice when the tube is removed. However, the latter phenomenon happened only rarely and could often be attributed to the air pressure in the tube being too low causing roots to grow over instead of in the soil (e.g. table 1, number 14). By reinsertion and reinflation of the tube they were pressed against the soil again, so the poor soil-root contact only existed during the period of photographing.

The effect of different tube pressures on the firmness of the minirhizotron hole and on root performance is shown in Table 2. In the sandy soil with low tube pressure, soil particles occasionally came off the wall or adhered onto the tube when it was deflated and removed; this occurred especially in the very wet subsoil. At the highest tube pressure this did not occur. With increasing tube pressure, soil-root contact was improved, while root hair formation varied. In the clay soil, having much more cohesion, no soil particles came off the wall in any of the treatments and soil-root contact was always good. Root hairs were only rarely seen. For both soils a tube pressure of about 0.125 MPa appears to be a proper value, although it may be somewhat lower for a clay soil.

When working with perennials such as trees or grasses, it is often desirable to study root growth dynamics for several successive years. In the humid temperate climate, soils may freeze dur-

Table 2. Effect of tube inflation pressure in the inflatable minirhizotron on firmness of the minirhizotron hole and on several root parameters of grass

Soil	Minirhiz. type[a]	Pressure (MPa)	Firmness[b]	Root/soil contact[c]	Root hairs[d]
Sandy	s	0.114	o	B/C	2
	s	0.118	o	C/D	0
	s	0.122	o	C	2
	s	0.126	r	C/D	0/1
	a	0.118	r	C	0
Clay	s	0.114	n	C/D	0/1
	s	0.118	n	D	0
	s	0.122	n	D	0
	s	0.126	n	D	0
	a	0.118	n	C	0/1

[a] s = stainless steel; a = aluminium.
[b] Firmness of the minirhizotron hole in the subsoil after deflating and removing the tube: n, r, o = ranging from no problems with firmness to rare or occasional coming off of soil particles from the wall.
[c] See Figure 4.
[d] 0 = absent; 1 = occasional; 2 = abundant.

ing winter, and rigid-wall minirhizotrons may break due to severe swelling of the soil. In the inflated tube system used during the winters of 1988/89 and 1989/90 both in a sandy soil and a heavy clay soil, no frost damage was apparent, and after the winter period the soil-root contact was still good.

This minirhizotron system, designed for the photographing of roots, also permits collection of roots from the holes after the tube is removed, e.g. for determination of N content of presence of V.A. mycorrhiza. Presently special systems (the so-called 'perforons') are in use for this purpose, in which a large number of small horizontal holes are made in a vertical wall in which roots can be sampled (Van den Tweel and Schalk, 1981). In these systems, however, root growth inevitably occurs in a gap environment.

Problems reported with the inflatable minirhizotron technique include 1. a possible cooling off of the tube surface in deeper soil layers due to heat convection through the air in the tube towards the cooler top compartment of the tube ('heat pump' effect), and 2. condensation of water on the endoscope when a cold endoscope is inserted into a relatively warm soil. This phenomenon can be avoided by warming up the endoscope with an electric hair dryer.

Conclusion

The inflatable-wall minirhizotron described here is an improvement of the commonly used rigid-wall systems. It ensures a proper contact between soil and minirhizotron wall, so that no roots can grow in voids or gaps along the tube. Therefore, the root growth dynamics observed will be more consistent with the natural undisturbed situation. Swelling or shrinking of soils, which results in gap formation in rigid-wall minirhizotrons, is now compensated for by the elasticity of the tube. As the inflatable part of the system is removed before the roots are observed, there is no barrier to bright-image recording. The roots are therefore much easier to distinguish than in the commonly used system.

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