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# An improved method for the isolation and the quantitative measurement of crop roots

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**Summary**

The isolation of crop roots from soil samples, making use of the dispersing action of carbon dioxide gas evolved by the reaction of added oxalic acid on carbonates in the soil has been described.

The roots are photographed in a thin layer of water against a matt black background, resulting in sharp negative pictures that can be used by a 'Quantimet' image analyser to measure length or other properties embodied in the photographs.

The methods are applied in a study of the rooting characteristics of potato crops in a rotation experiment.

## Introduction

Crop rotation experiments carried out on the uniform soil of the newly reclaimed Flevo Polder, showed marked differences in potato yields depending on the crop sequence. The potato yield in the short rotation: grass-seed – sugar-beet – potato was severely depressed compared to the yield in the rotation: winterwheat – flax – sugar-beet – summer barley – peas – potato. It seemed reasonable to attribute this impaired yield to some soil-borne influence, left by the preceding crop which had an adverse effect on the potato roots. In studying the actual cause of the yield depression a method was needed for the frequent investigation of undisturbed roots in the field during the growing season.

The foil method developed from the profile wall method (Reijmerink, 1964, 1973) could not be used frequently because the soil would be disturbed too much for its subsequent use in crop yield experiments. Moreover, this method did not allow for close inspection of the roots for damage or disease.

The methods for examination of roots using monoliths or soil cores (Schuurman & Goedewaagen, 1971) can be carried out more frequently than the foil methods

and will cause less damage both to the crop and to the soil. These methods necessitate however the removal of the soil from the roots by some washing procedure. The very heavy and compact clay soil from the polder was difficult to remove without subsequent damage to and even loss of part of the roots. To avoid such damage and breakage the washing process had to be carried out by gentle sprinkling with water but even so the soil tended to split into elements (prisms) that were hard to disperse. This splitting process often caused the roots between the prisms to break.

In attempts to overcome the difficulties experienced with the known methods, a procedure was derived that gave satisfactory results for our purpose during several years. Meanwhile the essentials of this procedure have been taken over by other workers in the same field and several useful aspects have been shown to exist.

## Methods and materials

*Isolation and preparation of the root samples*

Soil samples containing the roots to be studied are taken by a heavy soil auger (Schuurman & Goedewaagen, 1971) consisting of a cylindrical tube with an inside diameter of 7 cm and a length of 20 cm, fixed to a hollow shaft (Fig. 1a). The auger is hammered into the soil with a wooden mallet (Fig. 1b). The sample can

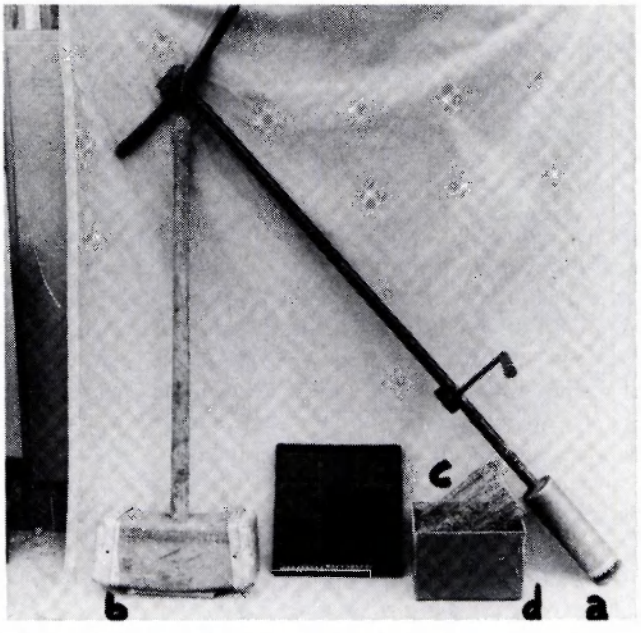


Fig. 1. Tools used for collecting and washing root sample. a: soil auger; b: wooden mallet; c: basket; d: washing box.

be forced out of the auger by a plunger located inside the tube, that can be moved by means of a rack and pinion arrangement built inside the shaft.

The soil samples (7 cm diameter and 10 cm length) from each hole are arranged, according to depth, in a gutter of 80 cm length which is closed on each side, for transportation to the laboratory. There, each sample is put into a basket made of coarse wire (3 mesh to 1 cm) in such a way that the roots lay parallel to the bottom wire screen (Fig. 1c). This basket is then suspended in a box (Fig. 1d) which is not much wider than the basket, but which is deep enough to allow all the soil to pass through the bottom screen and to gather underneath in the box. This box is filled with a solution of oxalic acid. The reaction of the oxalic acid with the calcium carbonate contained in the sample causes the formation of carbon dioxide and the bubbles of this gas help to dispers the soil aggregates and to clean the roots. This process, together with occasional shaking of the basket, will cause all the soil to fall through the bottom screen of the basket after some hours or overnight. Left behind are the roots, still between some clods and coarser soil components. The roots can easily been taken out by means of tweezers and washed free from adhering soil particles in a beaker of clear water.

The size and the type of the soil sample are obviously the indicative factors as to the dimensions of the basket and the box. In our case the basket measured 10 cm x 18 cm and 10 cm deep and the box 13 cm x 20 cm and 15 cm deep. The amount of oxalic acid required for effective dispersion of the soil depends on the kind of soil and the size of the box. Two table spoons (about 30 g) of oxalic acid for each box (containing about 3 litres water) proved to be most effective for the heavy sea-clay we had to handle. The problem posed by some soils not containing enough calcium carbonate can be overcome by the immersion of the not yet wetted soil sample in a solution of bicarbonate, preceding the treatment with oxalic acid.

*Photography*

The cleaned roots are transferred to a tray with dimensions of 25 cm x 25 cm and 3 cm deep, filled with a layer of approximately 6 mm water. This tray is painted matt black on the inner side and the bottom is covered by a sheet of alumina that is also painted matt black. In this paint a rectangle of 18.75 cm x 24 cm is scratched in thin (0.1 mm) lines and divided into 9 identical small rectangles of 6.25 cm x 8 cm.

Each root sample in turn, is arranged between the borders of the 18.75 cm x 24 cm rectangle in such a way that crossing of roots is avoided as much as possible. Potato roots with a total length of 6-7 metres can be arranged in this area with some care. The indices of the sample are printed on adhesive tape with a Rotex letter printer and fixed onto leaden strips, allowing easy arrangement onto the desired places in the tray containing water, outside the rectangle destinated to the roots.

The tray is illuminated with four 500 W daylight photo lamps. Each root sample is photographed with a 6 cm x 6 cm Rolleicord camera provided with a 2x magnifying close-up lens set at a distance of 35 cm, time ½ s and diaphragm

F = 16. The pictures are taken on Agfachrome 25 professional 'document copying' film. In this way detailed sharp negative pictures of the white roots on the matt black background were obtained and troublesome shadows were not created (Fig. 2).

*Measuring roots by a 'Quantimet 720' **

The 'Quantimet 720' image-analysing apparatus consists of an image producing part, an analysing part and a computing and registrating part. The image can be made by an optical instrument either a microscope or a projector and a special video camera.

The negative of the roots (showing dark roots on a clear transparant film) is projected and the enlarged projection is scanned by the special video camera. This camera converts the optical picture (built up by 625 x 800 picture points) into electronic signals. Dark points generate different signals from light points.

* Imanco, 1972: Quantimet 720. Instruction Manual.

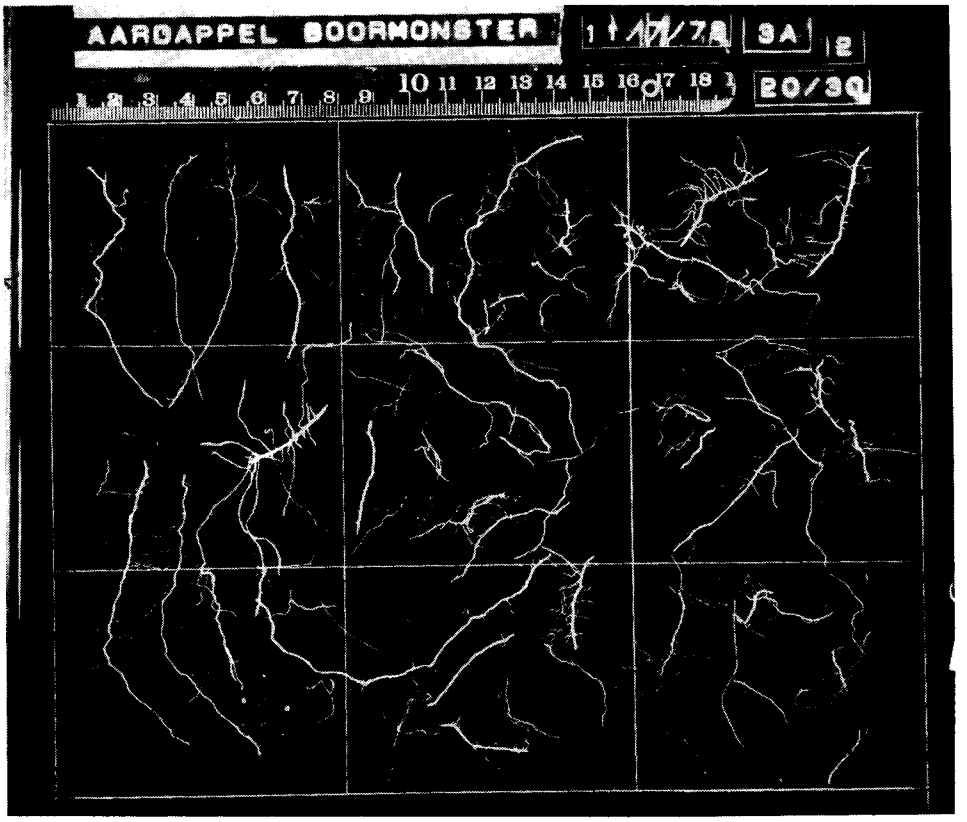


Fig. 2. Photograph of the roots from one sample.

A dark point or line on the light background will, of course, only be recorded if the diameter is at least as large as one picture point. To be sure that the roots with diameter of 0.1 mm are recorded, each picture point should cover not more than an area corresponding to 0.1 mm $\times$ 0.1 mm of the original roots and the total area covered by the 625 $\times$ 800 picture points must therefore be no larger than 6.25 cm x 8.0 cm.

As it was impossible to arrange the roots of the soil samples on that small surface, they were arranged on an area of 18.75 cm x 24 cm that was divided into 9 parts each 6.25 cm x 8.0 cm. The special video scanner was focused successively onto each of the 9 small rectangles and the results were combined.

The electronic signals, different for dark and light points, are introduced into a computer and by different programs this computer can measure several properties of the roots.

## Results

The development of potato roots was measured on two plots of a large-scale crop rotation experiment on uniform soil of the newly reclaimed Flevo Polder. One of the plots carried the rotation 3a, i.e.: winter wheat – flax$^+$ – sugar beet – barley – peas$^+$ – potatoes, and the other the rotation 5b i.e.: grass-seed – sugar-beet – potatoes ($^+$ indicates green manuring in the autumn with Italian ryegrass after peas and white clover after flax). Yield analyses have shown that in successive years the potato yield in the 3-year rotation (5b) was about 15 % lower than in the 6-year rotation scheme (3a).

In each plot, the samples were collected in 4 adjacent rows. These rows were chosen so that they did not border upon the track used by the spraying machines. The sampling started about 15 m from the beginning of the potato rows and progressed along the same rows every week during the months of June and July. Samples were taken close to a potato plant and because of the possibility of damage to adjacent plants during sampling, samples were only taken from every second plant in the row.

After the roots had been isolated, cleaned and photographed, the root samples were dried and the dry weight of each was measured. The mean weight of four identical samples was calculated and the results are shown in Fig. 3 for data obtained in 1977 and in Fig. 4 for 1978 (on the right side). The length measurements as computed by the 'Quantimet' and averaged for the 4 equivalent samples are also recorded in the Figs. 3 (1977) and 4 (1978) on the left side.

The potato plants were grown in ridges, made by earthing up the rows with soil from between the rows. Soil sampling started in 1977 from the level of the original soil surface, clearly distinguishable that year as a more compact original soil surface under the ridges. In that year, generally more root (in length as well as in weight) was found in the deeper zones (20-30 cm and 30-40 cm underneath the original soil surface) of the 6-year rotation 3a than in the corresponding zones of the 3-year rotation 5b. Such a clear and constant difference could not be seen in the zone 10-20 cm underneath the original soil surface. Weeks in which more

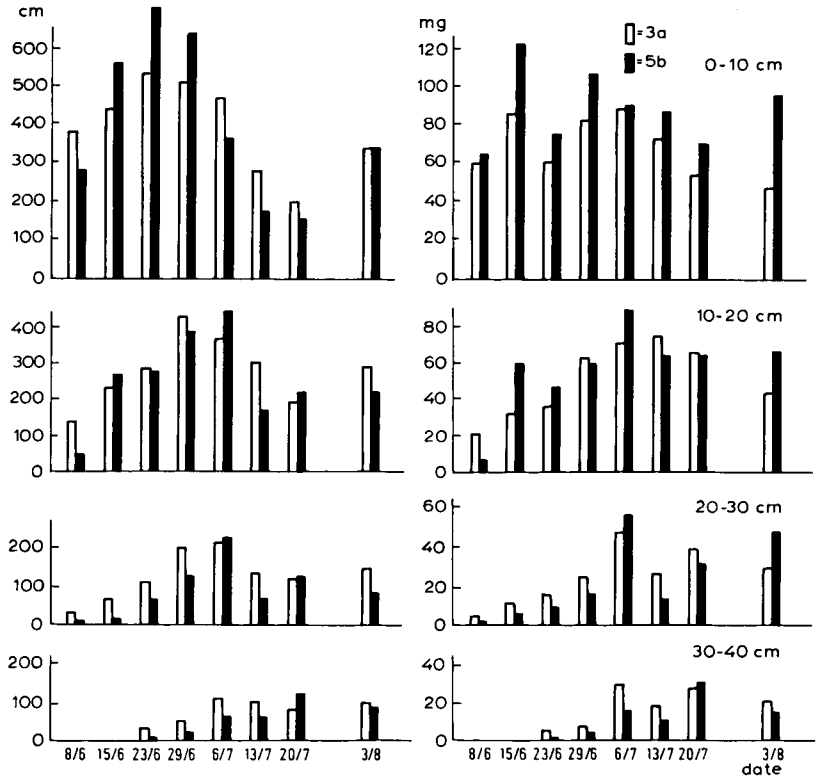


Fig. 3. Length in cm (left) and weight in mg (right) of potato roots collected from soil samples Ø 7 cm and 10 cm deep, from two rotations 3a (white bars) and 5b (black bars) at different dates in 1977. Average from 4 samples.

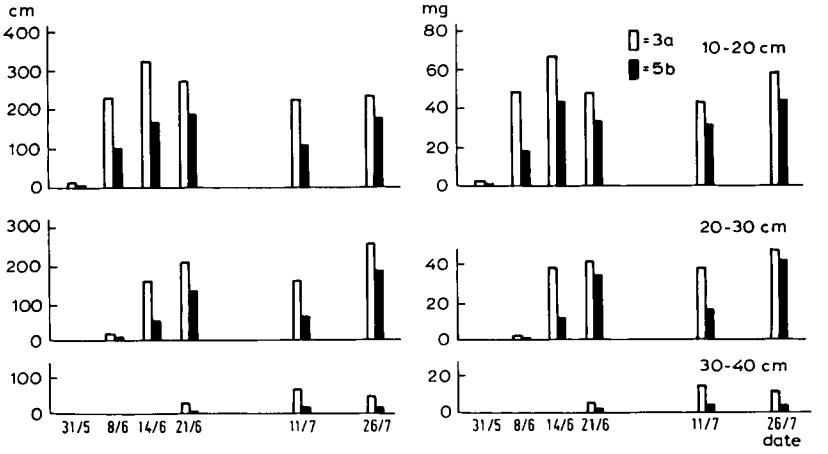


Fig. 4. Length in cm (left) and weight in mg (right) of potato roots collected from soil samples Ø 7 cm and 10 cm deep, from two rotations 3a (white bars) and 5b (black bars) at different dates in 1978. Average from 4 samples.

roots were found in rotation 3a than in 5b were often alternated by weeks in which the reverse was true. In this zone, consisting of the lower part of the ploughed soil the most brown, broken and dead roots were found, especially in the rotation 5b. In the upper zone 0-10 cm underneath the original soil surface, an initially greater root length in 3a was soon surpassed by the roots in 5b, the weight of the roots was from the beginning higher in rotation 5b than in 3a.

In 1978 (Fig. 4) the results of the preceding year were confirmed in so far as that in this year in all the zones below 10 cm underneath the original soil surface, more roots were shown in rotation 3a than in rotation 5b. In this year the soil in the ridges was so crumbled that it did not allow for differentiation into layers. The samples were taken from the whole ridge reaching from 10 cm above the original soil surface to 10 cm underneath that level. In this upper layer an early start was found in the weight of the roots from 3a above 5b at the first 3 sampling dates, but at the later determinations the opposite was true showing more roots in 5b than in 3a (Fig. 5).

## Discussion

Often it is not permitted to study the development of crop roots in experimental plots by the foil method (Reijmerink, 1973) or other profile wall methods (Schuurman & Goedewaagen, 1971; Böhm, 1979) as the digging of the necessary trenches would cause too much damage to the uniformity of the soil and to the crop. Taking resort to the study of the roots in soil-root samples collected by an auger, necessitates for the isolation and washing of the roots to get more information than an estimation of root length per unit soil volume as possible by the core break method (Schuurman & Goedewaagen, 1971; Böhm, 1979). The use of oxalic acid to help the dispersion of the heavy clay we had to handle turned out to give better results than other chemicals as pyrophosphates, sodium chloride, sodium carbonate (Böhm, 1979) or sodium oxalate as proposed earlier (Schuster & Stephenson, 1940). The formation of $CO_2$ gas bubbles generated by the reaction of the acid on the carbonates inside the soil lumps was very effective. It allowed for less mechanical agitation of the washing solution and for a coarser gauze for the passage of the soil particles and the collection of the roots. This method turned out to be very gentle and when the isolated roots were subjected to mi-

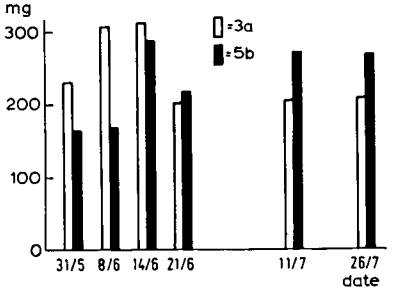


Fig. 5. Weight of roots in mg, collected from soil samples Ø 7 cm and 20 cm deep from the ridges in two rotations 3a (white bars) and 5b (black bars) at different dates in 1978. Average from 4 samples.

croscopic examination even most of the root hairs and fungal hyphae around the roots were found to be undamaged. The results obtained not only depend upon the methods used but also depend on the uniformity of the soil, the special properties of the plants investigated and the soil tillage.

The diameter and the length of the soil samples and the number of replicates obviously have to be adapted to the prevailing circumstances and to the accuracy required.

Not only are the lengths of the roots embodied in the photographs, but such parameters as diameter and rate of branching are also observed. These properties can be measured by the proper selection of the magnification to be used in scanning and by the programming of the computer of the 'Quantimet'.

We have not yet found a reliable method to distinguish between healthy and diseased roots. The colour of diseased roots generally results in diminished contrast with the matt black background of the tray in which they are photographed; evenso healthy young roots are sometimes quite transparent and also, therefore do not contrast as favourably against the background as the white opaque roots. Dead roots are usually so discoloured that they fade away during photography.

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