A study of the sugar beet root system by endoscopic techniques

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Introduction and Objectives

The study of root system development and morphology is useful for understanding the productive physiology of the crop species (Böhm, 1979). As soil does not allow a direct observation, the measure of the parameters related to root growth need fairly difficult procedures. Presently, in open field experiments, are used both destructive or non destructive methods (Taylor, 1987; Ehlers, 1996).

The destructive methods imply direct or auger-mediated picking up of soil samples; from these samples, root fragments are separated and measured.

The non destructive methods allow the study of the root apparatus through transparent screens placed in the soil. The most recent evolution of such systems are the "minirhizotrons", glass or plexiglass-made tubes installed in the soil, through which the observation by cameras or endoscopic probes is possible (Upchurch and Ritchie, 1983; Taylor, 1987; Ehlers, 1996).

The destructive methods have several disadvantage: they are labour-intensive and do not permit the repetition of the measures in the same point. Minirhizotrons allow faster and repeated observations; the main problem is the possible interference occurring at the soil-tube interface, where roots become visible. In this area, the roots find conditions different from the undisturbed soil and hence differences in the growth and evaluation mistakes can occurr (Taylor, 1987).

Using the minirhizotrons, it is important that the soil surrounding the tubes is not compacted during the laying operations. If this occurrs roots deviate from their natural path, leading to underestimate the density values. A limited adhesion between tube and soil leads to the opposite error and to overstimation of root growth (Brown and Upchurch, 1987).

Vos and Groenwold, 1987, and Upchurch, 1987, agree in suggesting that good levels of precision can be obtained with the use of small-bored tubes, with a 45° installation angle, uniformly placed in the soil and in sufficient number. In order to reduce the possibility of the interferences described above, the observations can involve only the portion of the tubes upward. In many cases, however, it is important to measure not the absolute values, but rather the differences between the different treatments (Mc Michael and Taylor, 1987).

Despite its physiological importance, the studies on root system development are few, probably because the technical difficulties in collecting significant data (Thomas, 1996).

As for sugar beet, data were collected only using the destructive methods (Windt, 1995; Ehlers, 1996; Märländer and Windt, 1996), while there is no knowledge of researches carried out with the non destructive methods, with the exception of Morselli and Biancardi, 1995.

In this paper are reported the results of experiments performed by the minirhizotron technique both on field plots with different levels of nitrogen fertilization and a three-year trial involving two sugar beet cultivars.

Nitrogen has a major influence on the productive characteristics of sugar beet. The nitrogen excess causes abundant leaf development, lowering of the polarimetric degree and of the processing quality (Lauer, 1995). On the other hand, the optimal nitrogen dose for the culture is difficult to be determined for the number and complexity of the factors to be considered (Allison and Clover, 1996). It is known that sugar beet is endowed with a more developed and deeper root system compared with other crops. It is also known that in the explored layers

the available amount of the different nutrients - and expecially of nitrogen - is not uniform (Peterson et al., 1979). The present work has the aim to study the relationships between the nitrogen levels potentially available for the plant at the various depths and the presence of roots at that depths.

The observation on the two cultivars aim to verify possible genotype-dependent differences in the root growth, able to explain for example their different degree of wilting during drought stress.

Materials and methods

Characteristics and installation of the minirhizotrons. For root observations, plexiglass tubes 4 m long, with an outer diameter of 4 cm and an inner diameter of 3.6 cm were used. To the buried end, the tubes were hermetically closed with a 2 cm-thick PVC disk, slightly smaller than 3,6 cm, glued to the tube inner wall. The external edges of the closed end were rounded in order to make the insertion in the ground easier.

On the tube outer wall, a longitudinal line was traced, for the correct orientation of the probe. The exact depth was given by transversal lines traced every 2.5 cm. For the tests, black Edding 780 pens were used.

The tube was left 10 cm protruding from the soil surface; in order to avoid access to light during the observations, the upper end was painted with black enamel for a length of about 15 cm. The upper closure was removable and obtained with a sheet of black polyethylene, kept in place with a rubber band.

The laying of the minirhizotrons has been made with a 45° angle, using a special platform with a metallic tube able to guide the auger at the right direction.

While making the holes in the ground, several systems were used to avoid the compaction of the walls. The most convenient system was the use of a hand-carried type Edelman auger with a 3.8 cm diameter and with 100 cm extensions, supplied with screw thread connections. The 4 cm diameter has been reached with a second auger 100 cm long and 3.6 cm wide, supplied with two blades protruding 0.2 cm. By rotating the reamer, the cavity can be extended removing the layer probably compressed by the action of the first auger; the excess soil is collected at the inside.

The minirhizotrons were placed immediately after the excavation, with the longitudinal line upward, and leaving a 10 cm piece protruding from the ground.

Observation system. The measurements were performed with an optical fiber probe Olympus IF 1303, 6 m long and supplied to one end of a wide-angle objective, of lighting outputs and, to the other end, of eye-piece and of the focussing system. To the eye-piece was applied a Sony video camera DXC-1079, connected with a 9-inches color monitor and a Video 8 recorder. These instruments were placed, along with the light source and the camera power supply, in a home-made small hand-driven cart suitable for open field use. All the described instruments have a total weight of less than 80 kg, including a 600 W power generator.

The observations were performed by manually moving the probe from the bottom upward, at about 5 cm/s speed, and checking on the monitor the focus and the alignment to the longitudinal line of the tube.

The shots concerning the upward part of the tube allowed the observation of a 3.5 cm arc of circumference on the soil wall; this is possible because both the small dimension of the probe and of the wide-angle objective.

The roots visible from the recorded images were drawed on diagrams (Fig.1) on which, by using a map measurer, it was calculated the length at the different depths (Upchurch, 1987; Glinsky et al., 1993).

The length of the utilizable tube (387 cm) was divided into 8 sectors, each 50 cm long, except the last one that was 37 cm long; taking into account the tube slope, each 50 cm sector

corresponds to a 35 cm layer of soil. The maximum depth attainable with the described instrumentation is about 273 cm from the soil surface.

Data processing. The data obtained, initially expressed as cm of root per tube (L), were transformed into root length density (RLD), expressed as cm of root per cm^3 of soil. This transformation is necessary to make the data set independent from the instrument and the tubes used. It was assumed that the roots visible through the transparent wall are enclosed in a 0.1 cm thick layer of soil,outside the tube itself (Voorhees, 1976; Glinski et al., 1993).

The volume of soil, to whom the root length is referred, is equal to a parallelepiped with a basal area of 3.5 cm x 0.1 cm = 0.35 cm^2. Therefore, for each tube, the maximum volume the roots can explore is 0.35 cm^2 x 387 cm = 135 cm^3. If higher thicknesses are considered, as proposed by Sanders and Brown (1978), the densities proportionally decrease.

By measuring the length L for every 50 cm of tube, and by referring this value to the corresponding soil volumes, the RLD value is obtained, for each 35 cm layer of soil (Beyrouty et al., 1988).

In order to represent the root development as a function of time, it is useful to express L in km per plant. Considering that the plant density was 10 plants/m^2, it is possible to exactly calculate the development of the root system per unit area.

Nitrogen fertilization. To determine the influence of nitrogen on the root growth, it has been used a normally cultivated area, not fertilized with nitrogen since 1983. This choice was due to the necessity of maintaining effective levels of nitrogen deficiency at the different depths, and a sure differentiation between the treatments.

To avoid all the interactions, on the whole testing area it was established a fully normal availability of all other elements. The test performed in 1994 was organized in a randomized blocks design, with 18 plots (three nitrogen levels by six repetitions). The three nitrogen amounts (0, 100 and 200 kg/ha) were manually distributed as ammonium nitrate, before the sowing.

The layering of the tubes, carried out immediately after sowing, consisted of two neighbouring repetitions. Because the high data variability (Upchurch and Ritchie, 1983), six tubes per plot were employed, on three near rows of beets and the corresponding interrows; between the first and the sixth tube the distance was 112.5 cm. On the 36 tubes, 10 observations were performed every two weeks beginning from May 23rd. On June 20th, the leaf area was measured on 40 plants per treatment. In order to avoid interferences, weeds were manually eliminated weekly.

Varietal differences. The two cultivars chosen, Rizor and Cremona, are largely employed in Italy, and are endowed with different agronomical characteristics; they have been selected after preliminary tests including 10 more genotypes.

For each variety, four tubes, placed as previously described, were used. Also in this case 10 observations each year were carried out. With the aim to make the root dynamics more comparable in the three years test, only the six observations performed in the same date were considered. All the agronomical tests were conducted at Rovigo, Italy.

Results and discussion

Nitrogen fertilization. The soil analysis in 1992 and in 1994 confirm the progressive reaching of a nitrogen deficiency at all the depths (Table I). This is confirmed by the productive data, as a 200 kg/ha of nitrogen supply involves -differently from the years 1985 and 1992- a significant increase in sucrose production compared to the control treatment (Table II).

The growth of the root system is shown in Figure 2. The length of the roots per plant rapidly increases until June, then declines slowly until the half of September and more sharply thereafter. The differences between the curves "zero Nitrogen" and "200 Nitrogen" are around 30%, and are significant for three dates. The "100 Nitrogen" curve has an intermediate trend.

The length of the roots are inversely correlated to the commercial root weight at harvest. If such differences proportionally hold also in previous periods, and considering the ratio total length/weight of the commercial root, the differences between "zero Nitrogen" and "200 Nitrogen" are significant in 8 out of 10 observations.

The higher root development in the "zero Nitrogen" plot is not apparently due to a water shortage. Despite of this, the "zero Nitrogen" test showed during the life cycle a lower leaf development compared to the "200 Nitrogen" test (LAI 4.3 and 6.4, respectively), that in turns should have involved a proportionally lower water loss by canopy transpiration, and root development is higher.

In Figure 3, the root densities in the different layers in 6 observation data are shown. It can be noted a higher development in depth for the fertilized plots at the last three observations. This is probably due to a higher water need. In the same figure, it can be observed the relative lack of roots in the most superficial layers, contrasting with reports from different environments and using other approaches made by other authors (Windt and Märländer, 1994).

Variety differences. The tests were performed using a factorial design (2 cultivars x 3 years x 6 observations). Variance analysis (Table III) did not show significant differences between the varieties. On the contrary, significant differences were found between the years and the observation dates, as well as the interactions variety x year and observation x year. By calculating the variance components for each factor (Wolf, 1995), the prevalence of the factor "year" on the factor "observation" can be deduced. The factor "variety" has little influence.

Figure 4 show the density profile reached by the two cultivars in 1994. The differences seldom are significant when single observations are considered. Figure 5 shows the evolution of the average length of the roots during the three years. The root development is very different, probably due to a different climatic trend. Infact, while 1994 was characterized by some drought periods, in the other two years rainfalls were abundant in the whole growing season, causing compaction of the soil during the spring. Because the alternate positive and negative factors, the sucrose yield did not show significant differences between the three years.

In Figure 6, the correlation between the root length reached in the three years, and the growing degree days, calculated with a 3°C basic temperature (Jaggard et al., 1996), is shown. The poor root development in the last two years might also be due to the relatively low temperatures.

Conclusions

The use of the minirhizotrons allows new approaches in the analysis of deep root systems. The described system provides data more representative than those obtained with destructive methods; this is especially true if care is taken to minimize the different error sources, mainly generated by a not perfect contact of the soil to the outer wall of the tubes. It must however be pointed out that the correspondence between the two methods is limited, because the analysis are performed with totally different approaches. Once overcome the problem of the data variability, the minirhizotron system allow a more precise investigation of questions related to the availability of water and of nutritive elements in the soil layers explored by the roots.

From the data presented, it results that root development is dependent upon nitrogen fertilization and climate, while the varietal effect appears negligible. However, it can be made the hypothesis that some of the problems of production or extractive quality, frequently uncertain, could actually be related to the extension in length and depth of the root system, or to the existence of soil layers with a different water availability and nitrogen content.

The depth reached by sugar beet roots in non-limiting water conditions can be beyond 270 cm; it is conceivable that this depth can be even exceeded in drought conditions. It is therefore possible that the currently used systems of soil sampling need to be modified, in order to get results correctly representing all the soil layers reached by the roots.

References

Allison M.F. and Clover G.R.G., 1996. Nutrient uptake by the sugar beet fibrous root system. Proc. IIRB, 59,165-174.

Beyrouty C.A., Wells B.R., Norman R.J., Marvel J.N. and Pillow J.A., 1988. Root growth dynamics of a rice cultivar grown at two locations. Agronomy J., 80, 1001-1004.

Böhm W., 1979. Methods of studying root systems. Springer Verlag, Berlin, 188 p.

Brown D.A. and Upchurch D.R., 1987. Minirhizotron: a summary of methods and instruments in current use. In: Taylor H.M. (ed.). Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics. ASA Special Pubblication, 50, 15-30.

Ehlers W., 1996. Methoden zum Studium der Wurzelentwicklung der Zuckerrübe. Proc. IIRB, 59, 153-164.

Glinsky D.S., Keith J.K. and Carrow R.N., 1993. Comparison of reporting methods for root growth data from transparent interface measuraments. Crop Science, 33, 310-314.

Jaggard K.W., Dunham R.J., Brown K.F. and Werker A.R., 1996. Season and soil-type effects on sugar beet root development. Proc. IIRB, 59, 213-228.

Lauer J.G., 1995. Plant density and nitrogen rate effects on sugar beet yield and quality early in harvest. Agronomy J., 87, 586-591.

Märländer B. and Windt A., 1996. Entwicklung des Faserwurzelsystems und dessen Bezug zu Nährstoffaufnahme und Wachstum der Zuckerrübe. Proc.IIRB, 59, 187-198.

Mc Michael B.L. and Taylor H.M., 1987. Application and limitation of rhizotron and minirhizotrons. In: Taylor H.M. (ed.). Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics. ASA Special Pubblication, 50, 1-13.

Morselli A. e Biancardi E., 1995. Studio dell'apparato radicale della barbabietola da zucchero mediante tecniche endoscopiche. L'Industria Sacc.Ital., 88, 147-151.

Peterson G.A., Anderson F.N., Varvel G.E. and Olson R.A., 1979. Uptake of ^{15}N-labeled nitrate by sugar beets from depths greater than 180 cm. Agronomy J., 71, 371-372.

Sanders J.L. and Brown D.A., 1978. A new fiber optic technique for measuring root growth of soybeans under field conditions. Agronomy J., 70, 1073-1076.

Taylor H.M., 1987. Minirhizotron observation tubes: Methods and applications for measuring rhizosphere dynamics. ASA Special Pubblication, 50, Madison, WI, 143 p.

Thomas T.H., 1996. Form and function of the sugar beet fibrous root system; its importance to crop productivity. Proc. IIRB, 59, 143-152.

Upchurch D.R. and Ritchie J.T., 1983. Root observations using a video recording system in minirhizotron. Agronomy J., 75, 1009-1015.

Upchurch D.R., 1987. Conversion of minirhizotron-root intersection to root length density. In: Taylor H.M. (ed.). Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics. ASA Special Pubblication, 50, 51-65.

Voorhees W.B., 1976. Root elongation along a soil-plastic container interface. Agronomy J., 68, 143.

Vos J. and Groenwold J., 1987. The relation between root growth along observation tubes and in bulk soil. In: Taylor H.M. (ed.). Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics. ASA Special Pubblication, 50, 39-49.

Windt A. and Märländer B., 1994. Wurzelwachstum von Zuckerrüben unter besonderer Berücksichtigung des Wasserhaushaltes. Zuckerindustrie, 119, 8, 659-663.

Windt A., 1995. Entwicklung des Feserwurzelsystems der Zuckerrübe sovie dessen Beziehung zum Wachstum der Gesantpplanze und der Nährstoffanfnahme. Cuvillier Verlag, Göttingen, 101 p.

Wolf I., 1995. Sorte und Sortenwahl bei Zuckerruben und derem Wechselwirkung zu Umwelt und Qualitätsbezahlung. Cuvillier Verlag, Göttingen, 117 p.

Year	Depth (cm)	Ammonium N (ppm) a	Nitrate N (ppm) b	Mineral N (ppm) a+b	Organic N (ppm) c	Total (ppm) a+b+c
1992	0 - 50	5.1	8.0	13.1	19.3	32.3
	51 - 100	2.5	3.7	6.2	15.6	21.8
	101 - 150	1.9	13.1	15.0	6.5	21.5
	151 - 200	3.6	21.1	24.7	8.1	32.9
	201 - 250	2.4	13.4	16.8	6.7	23.5
	251 - 300	6.7	3.5	10.3	9.1	19.4
1994	0 - 50	2.8	16.3	19.1	9.8	28.9
	51 - 100	2.7	9.0	11.7	9.5	21.2
	101 - 150	1.7	4.9	6.7	4.3	11.0
	151 - 200	2.4	3.3	5.7	2.5	8.3
	201 - 250	2.7	2.5	5.2	0.8	6.0
	251 - 300	6.0	1.0	7.0	11.9	18.9

Table I - Concentration of some nitrogen components in the soil at increasing depth. The extraction was performed with Electro ultra filtration.

Year	N fertilizer rate (kg/ha)	Root yield (t/ha)	Sugar conc. (%)	Sugar yield (t/ha)	Ext. sugar yield (1) (t/ha)	K	Na	α -N	Purity (2)
						——— mmol/100°S ———			
1985	0	72.4	18.9	13.6	12.0	27.4	5.2	9.1	93.41
	120	83.3	16.4	13.7	11.4	31.8	14.5	21.8	89.67
1992	0	81.2	17.9	14.5	12.8	28.1	4.4	13.6	92.76
	200	85.3 *	17.0 *	15.1	13.1	28.1	8.4 **	18.8 **	90.90 **
1994	0	60.2	15.51	9.3	8.5	21.4	5.6	4.9	94.81
	200	79.5 **	14.6 **	11.6 **	10.2 **	24.6 **	11.9 **	13.5 **	91.97 *

Table II - Effects of nitrogen fertilization on the productive and qualitative traits of sugar beet. The tests were performed on the same soil not manured since 1983. 1985 data were not processed.
(1) and (2): according to Wieninger - Kubadinov and Carruthers - Oldfield, respectively.
*, **: significant P=0.05 and P=0.01 levels, respectively.

Observations	1994			1995			1996		
	R (1)	C (2)	M (3)	R	C	M	R	C	M
	----- km/plant -----			----- km/plant -----			----- km/plant -----		
1	6.0	5.5	5.7	1.9	1.4	1.7	1.4	2.1	1.8
2	8.2	6.6	7.4	2.6	3.5	3.1	2.8	2.6	2.7
3	6.2	4.5	5.4	2.8	3.1	3.0	3.7	3.4	3.5
4	7.5	5.8	6.6	2.9	4.1	3.5	4.6	4.3	4.4
5	4.8	1.9	3.3	4.5	5.9	5.2	3.5	3.9	3.7
6	9.4	8.1	8.7	5.0	6.4	5.7	2.9	3.4	3.2

Source of variation	df	mean squares	variance components (%)
Cultivar	1	2.06	0.1
Observations	5	21.30 **	13.3
Year	2	123.93 **	41.1
C x O	5	0.33	0.0
C x Y	2	18.08 **	11.1
O x Y	10	15.54 **	28.2
O x C x Y	10	1.13	0.0
Error	108	1.54	6.2

Table III - Root length of 2 sugar beet cultivars on 6 stages of the growing (upper panel) and analysis of variance (lower panel); years 1994, 1995 and 1996.
(1): Rizor; (2): Cremona; (3): Mean. *, **: significant at P=0.05 and P=0.01 levels, respectively.

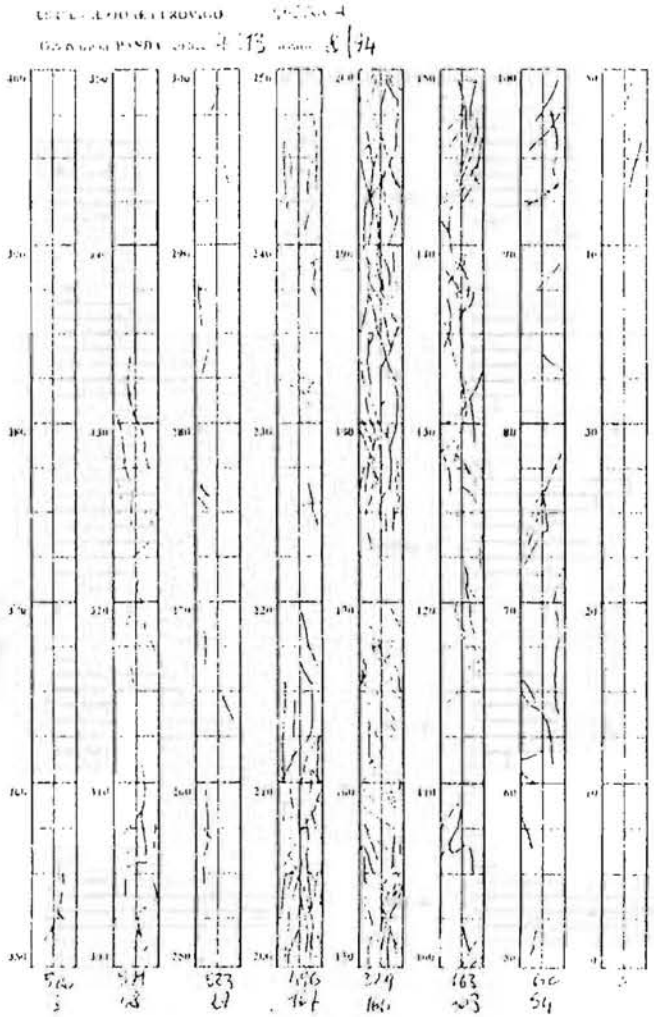

Figure 1 - Diagram showing the root system as visualized by the camera images. The printed numbers indicate the distance, in cm, from the 0 point. In order to obtain the depth, it is necessary to multiply by 0.71. The most superficial part is on the right, ranging from 0 to 35 cm in depth. The measure was performed on August 30th, 1994.

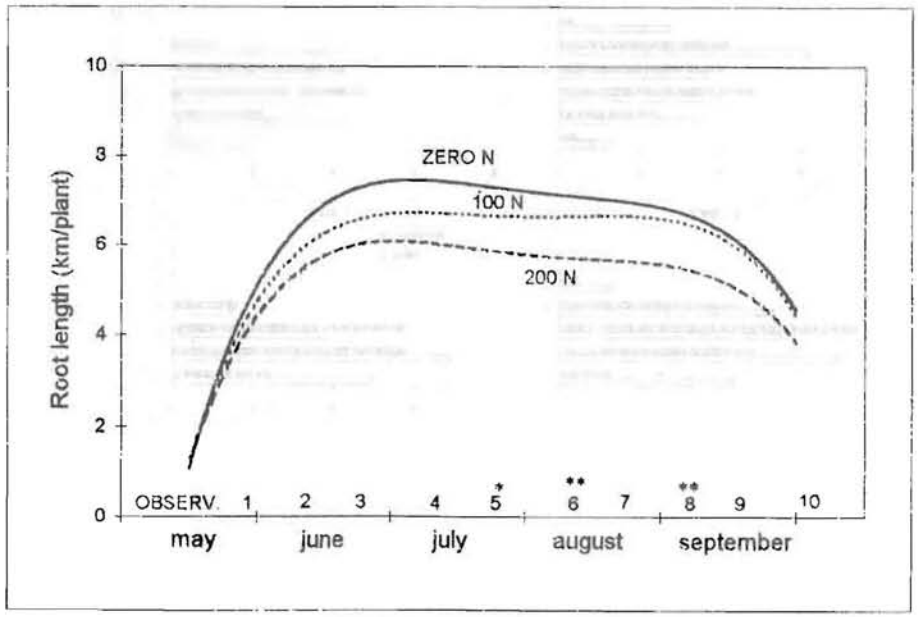

Figure 2 - Root lenght development in the different nitrogen treatments. For some observations, the differences between the "zero nitrogen" and "200 nitrogen" plots resulted significant (* for P=0.05, ** for P=0.01). The trial was carried out in 1994.

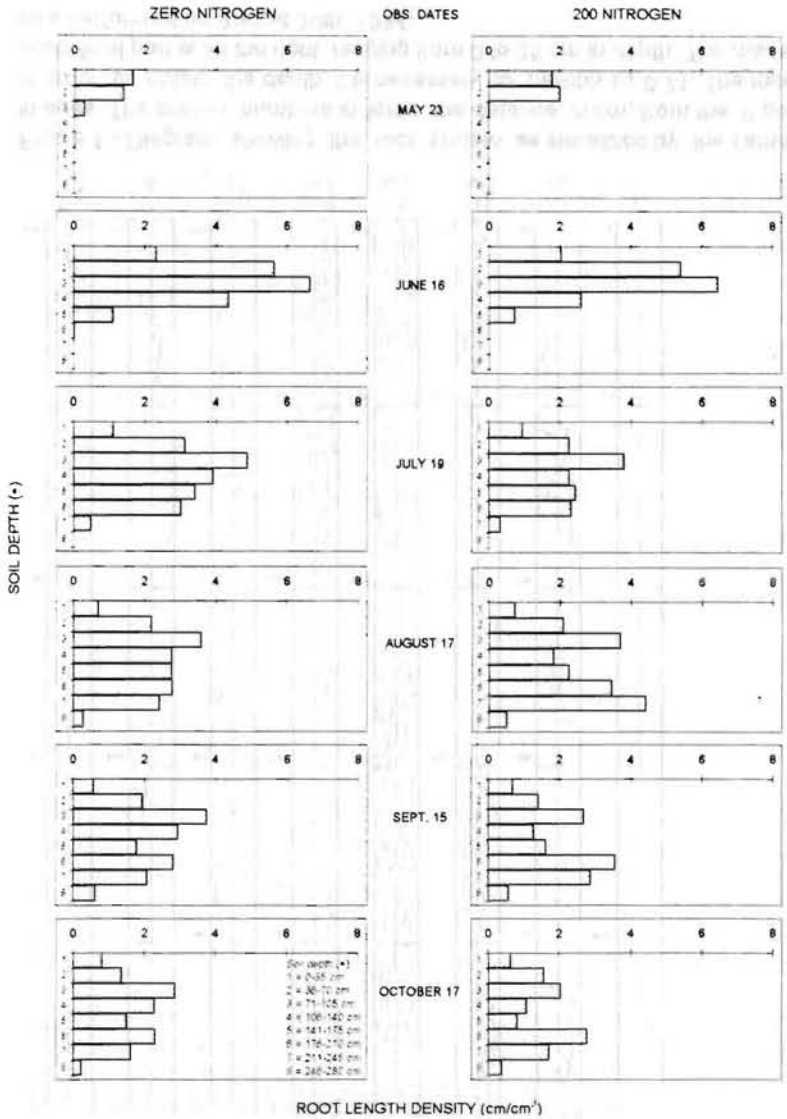

Figure 3 - Root density (RLD) at two fertilization levels, measured at 6 different observation dates. Year 1994.

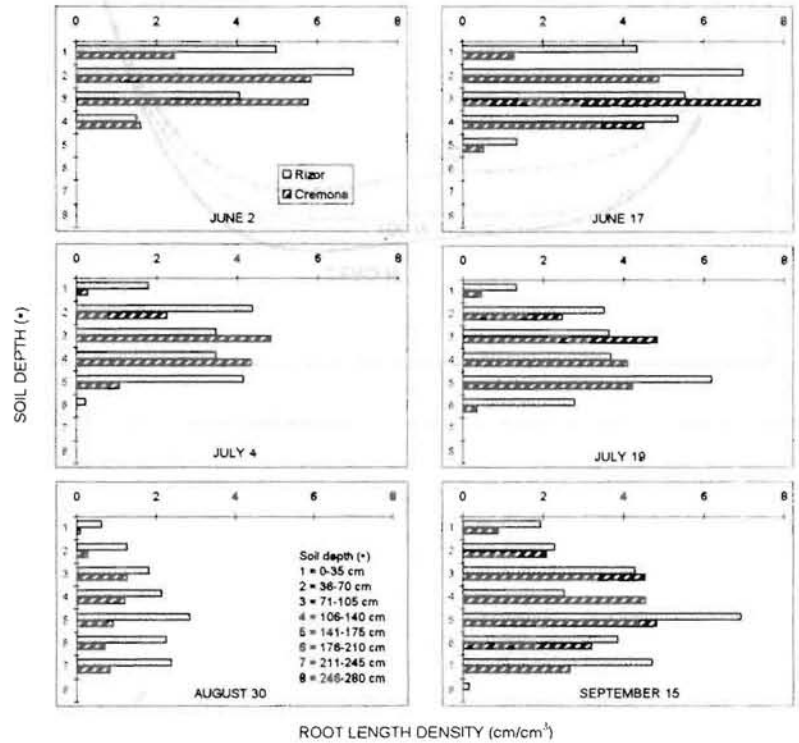

Figure 4 - Root density of the two cultivars at the different depths in six observation dates. Year 1994.

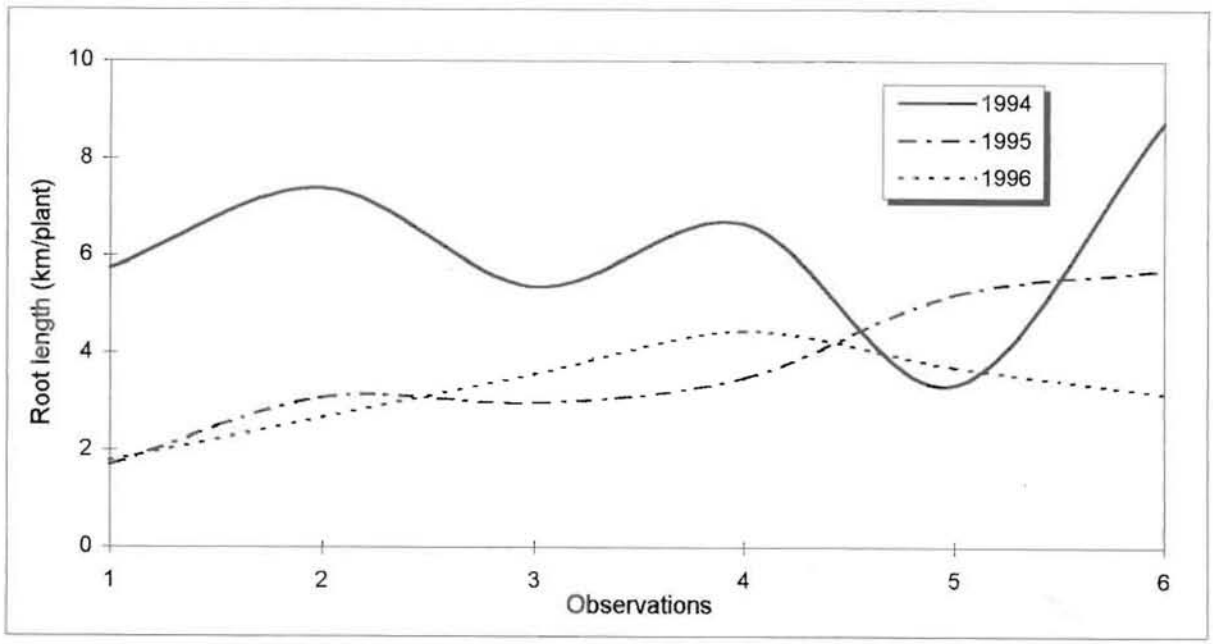

Fig. 5 - Evolution of the root length during the growing season.

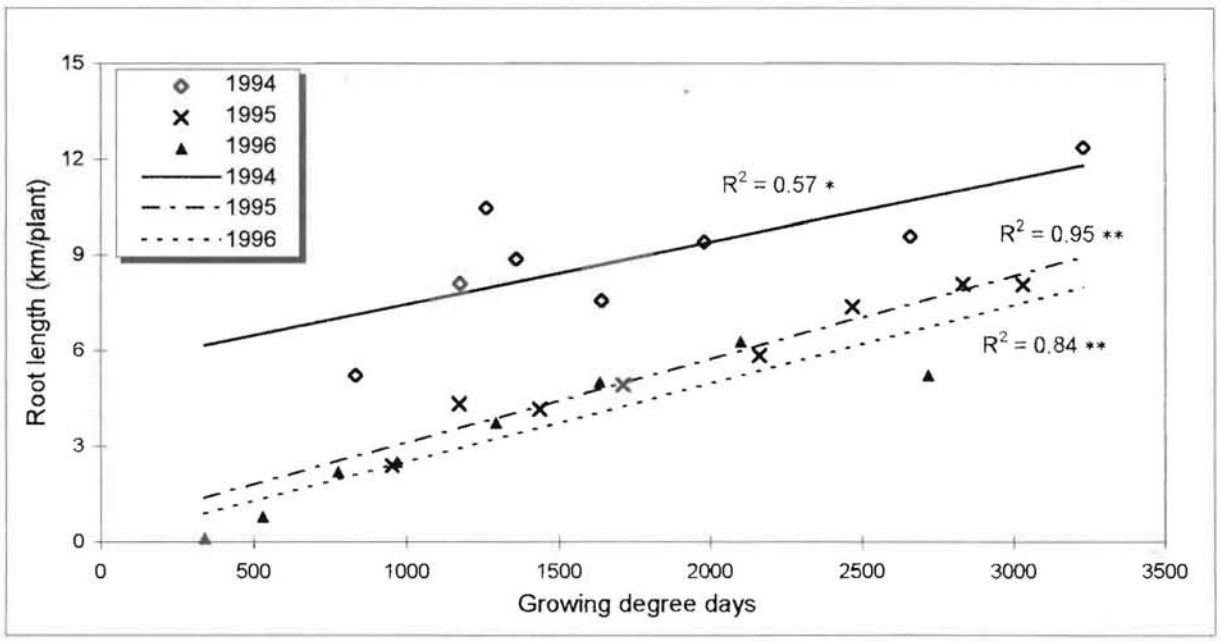

Figure 6 - Correlation between the root length and the growing degree days, calculated with basic temperature of 3°C.
*, **: correlation significant for P=0.05 and P=0.01 respectively