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Investigation of grapevine root distribution by *in situ* minirhizotron observation

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Summary

Root observations of *Vitis berlandieri* x *Vitis riparia* were conducted in two experimental sets using minirhizotron technique. Experiment 1 was a field experiment carried out on a 12 years old Riesling/5C vineyard. On six plants three minirhizotrons were installed at different angles (90°, 60°, 45°) and two directions per tube were used for observation. The maximum of root length density (RLD) was found in soil depths of 600-800 mm with high variation mainly due to plant x angle interaction. Observation direction did not influence the estimates of RLD. The installation angle of the tubes did not lead to any consistent effect on root observation. Experiment 2 was a pot trial of six pots with four vines each. Tubes were installed horizontally. RLD in the pot experiment according to the monolith method and the estimated RLD according to the minirhizotron method did not correlate, so the quantification of *Vitis* RLD distribution using minirhizotron is difficult.

Key words: root observation, *Vitis*, measurement accuracy, root distribution.

Introduction

The standard book on root research by Böhm (1979) starts with the following comment: "Root research under natural field conditions is still a step-child of science. The reason for this is primarily methodological. The known methods are tedious, time-consuming and the accuracy of their results is often not very great."

This is still true, although 30 years have passed since then. To overcome these problems, new methods were developed as for example the minirhizotron method which was already mentioned by Böhm (1979). In the following years many root studies have been carried out using minirhizotron techniques for various plant species, mostly annual plants (Upchurch and Ritchie 1983, Vos and Groenwold 1983, Meyers and Barrs 1985, Box and Ramseur 1993), rarely with woody plants (Buckland *et al.* 1993), to investigate the methodological aspects of this method. But to our knowledge only one experiment with *Vitis* is reported in a research note (McLean *et al.* 1992).

One problem of root observation is the high variability of root distribution (Böhm 1979). The root system of V*itis* is sparely distributed; it has lower RLD compared to annual plants usually examined with minirhizotrons, so the question is how accurate the minirhizotron observation of *Vitis* is. Furthermore it has been noted that the minirhizotron method has to be evaluated for every crop and even for the different installation techniques (Vos and Groenwold 1983, Box and Ramseur 1993, Smit *et al*. 2000). Even the recommended installation angle is reported controversially (Bragg *et al.* 1983, De Ruijter *et al.* 1996). As minirhizotrons can be used for the observation of roots for several years, this is a favourable method especially for the investigation of root dynamics (Smit *et al.* 2000). As opposed to root dynamics, this experiment addresses the issue of root distribution. The dynamic aspects of this investigation have been published in Lehnart *et al.* (2008).

The above cited preface (Böhm 1979) continues with the words: "Many research workers have been discouraged by doing such root studies." The aim of our experiment was to investigate if the minirhizotron method is able to enhance root observations of *Vitis i.e.* to encourage researchers for root studies. Therefore the methodological aspects of the minirhizotron method were investigated. Furthermore this trial was to examinate whether the results of the minirhizotron observation of *Vitis* according to the distribution of the roots are similar to the results found by the monolith method.

Material and Methods

Field experiment

Experimental design: The field experiment was carried out in 1996 in the Rheingau, Germany, (50°N, 8°E) in a vineyard of *Vitis vinifera* Riesling grapevines on 5C *(Vitis berlandieri* x V*itis riparia*) rootstock planted in 1988. Vines were trained to one vertically positioned cane and spur pruned to 10-12 buds m^{-2}. Spacing was of 2.5 m^2 per vine plant with permanent green cover in every row. The soil type was a rigosol, the A horizon (0 - 30 cm) consisted of silty sand with a brownish black colour and a pH of 7.4. The B horizon (30 - > 130 cm) also consisted of silty sand, was slightly compact, of brownish colour and with low carbonate content. The gravel fraction in the soil was < 5 %.

Minirhizotron root observations: The minirhizotron tubes were inserted in summer 1994, by first drilling a hole with a spiral auger. The clear acrylic tubes were 1,300 mm long with an external diameter of 60 mm. The investigation was carried out on six single vines here named with letters A-F. Three minirhizotrons were inserted for each plant. Each minirhizotron has been arranged at another side of the vine plant, one vertically at a distance of 100 mm from the plant, one at an angle of 60° to the soil

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surface and at a distance of 500 mm to the vine and the last one at an angle of 45° and a distance of 560 mm (Fig. 1). The parts of the minirhizotrons extending above the soil surface (200 mm) were capped to prevent the entry of light and water. To assure that root growth was not confounded with the appearance of roots from weed species, areas around minirhizotrons were kept mechanically weed free.

Roots have been observed with a BTC-2 camera (Bartz Technol.) which was mounted on a slight support. The visible frame was of 19.0 x 14.2 mm. The number of roots in the observation rectangle was counted according to a modified intersection method: Each root passing the whole window from one side to another was one count, independent of the length of the root, branches and roots intersecting only one or even no window side were a half count. According to BUCKLAND *et al.* (1993), the root number was converted to RLD assuming the roots would have grown through the volume of the tube, using the following equation and definitions: $RLD = N / (p \cdot \pi \cdot r \cdot L)$; N = root number, p = proportion of tube surface that was observed (b/U), r = radius of the tube (30.0 mm), b = circle segment of the observation window (19.0 mm), U = perimeter of the tube (188.5 mm), L = length of the observation window (14.2 mm).

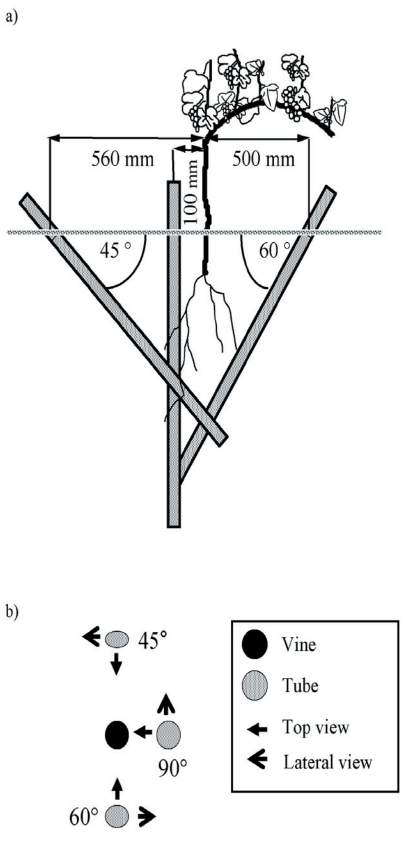

Fig. 1: Installation of the three minirhizotron tubes at different angles (45°, 60° and 90°) at distances of 100-560 mm of the vine and the camera observation directions "top" and lateral" view. Installation sen from **a**) the side **b**) above.

Root counts are assumed to be a better parameter for estimating RLD than visible root length because they are less influenced by conditions at the interface and because of their independence of properties expressed by the root after that root has touched the tube (SMIT *et al.* 2000). The equation above to convert root numbers into RLD can be written as $RLD = c \cdot N$ with $c = (p \cdot \pi \cdot r \cdot L)^{-1} = 7{,}410$ [mm^{-2}] in this experiment.

Root observations were made with the camera top view to the vine and laterally in 90° to this direction with the aim of comparing these two sights. Images of 76 locations per sight along each tube were recorded in April 1996.

S t a t i s t i c a l   a n a l y s i s : Prior to statistical analysis, the RLD was combined for soil layers of 100 mm thickness. Due to different slope (90°, 60°, 45°), 7, 8 or 10 windows were calculated to the RLD mean of a 100 mm layer. To detect significant differences between the independent variables ("plant", "angle", "sight") and their interactions a multiple ANOVA with the Tukey test at a significance level of 95 % was applied. The portion of variance was calculated by dividing the sum of squares of the effect through the total sum of squares. As for 45° sloped tubes only a soil depth of 800 mm was reached, no soil layers > 800 mm were used for these calculations. The confidence interval $L_{90\%}$ resp. $L_{95\%}$ was calculated with student t according to SACHS (1992).

P o t   e x p e r i m e n t

E x p e r i m e n t a l   d e s i g n : Three-year-old Kober 5BB vines (*Vitis berlandieri* x V*itis riparia*) grown in a nutrient-film-technique installation were used for this pot trial. In 60 L pots (280 x 380 x 580 mm), four vines per container were potted at a distance of 150 mm in perlite (an inherent material, 1 mm thick) directly on the horizontal minirhizotron tubes (Fig. 2). The vines were fertilized with nutrition solution which contained 0.1 % Flory Basisdünger 1®, 0.003 % Flory 10®, 0.003 % $CaSO_4$, 5 ppm Fetrilon. Six containers were used in this trial; the nitrogen fertilization level differed from 0-100 $mg \cdot N \cdot L^{-1}$ between the containers.

M i n i r h i z o t r o n   r o o t   o b s e r v a t i o n s : For the pot experiment, the same tube type was used as for the field experiment (see above). The minirhizotrons were inserted horizontally through the containers, directly under the vine plants; the extending parts were capped (Fig. 2). For camera technique, root number counting method and conversion from root number to root length density see the field experiment section.

Root observations were made on the left and on the right side at 90° from the vine plant. The aim was to com-

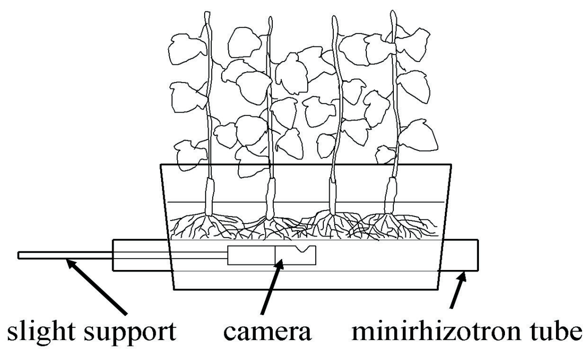

Fig. 2: Illustration of the pot experiment with horizontally arranged minirhizotron tube.

pare minirhizotron observations with harvesting roots using the monolith method. On 40 locations per side along each tube, at the end of the trial (82 d after planting) roots were counted and substrate monoliths (60 x 140 x 70 mm) at the left and right side of the tubes were dug up from the pots. Roots were separated with a hose using a 2 mm sieve. Root length per monolith was calculated with the grid intersection method according to TENNANT (1975) using a grid of 20 x 20 mm and converted into RLD with the volume of the monolith.

Statistical analysis: The mean RLD according to minirhizotron observation was calculated for the length of the corresponding monolith (140 mm or 10 windows respectively). The following linear regression was tested with a significance level of 95 %.

Results and Discussion

Variation of RLD: RLD distribution differed strongly between the observations per vine plant (Fig. 3) and also between the examined vine individuals (Fig. 4). This high variation in root observation is well known (BÖHM 1979); as a result of high variability a high number of observations are required to get reliable data. The confidence interval $L_{95\%}$ of RLD in a soil depth of 500 to 800 mm with six replicates is ± 10000 m m^{-3} due to the high standard deviation of about 4000 m m^{-3} (Fig. 4, Fig. 5). So $L_{95\%}$ was of 200 % of the mean RLD. To detect differences of 50 % of the means at this significance level 36 replicate observations are necessary. Even at a significance level of 90 % still 14 replicates are necessary (Fig. 4). UPCHURCH

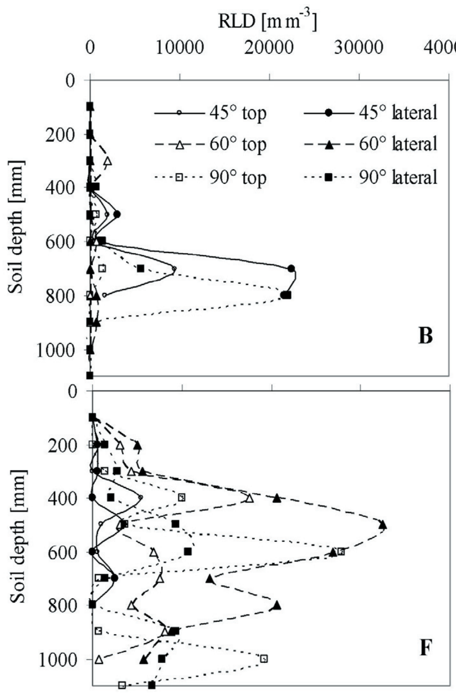

Fig. 3: Profiles of root length density (RLD) for two example vines (**B**, **F**) as observed in the three minirhizotrons with angle 45°, 60° and 90° and the observation directions "top" and "lateral" view. Each value was calculated from 10, 8, 7 single windows of the camera.

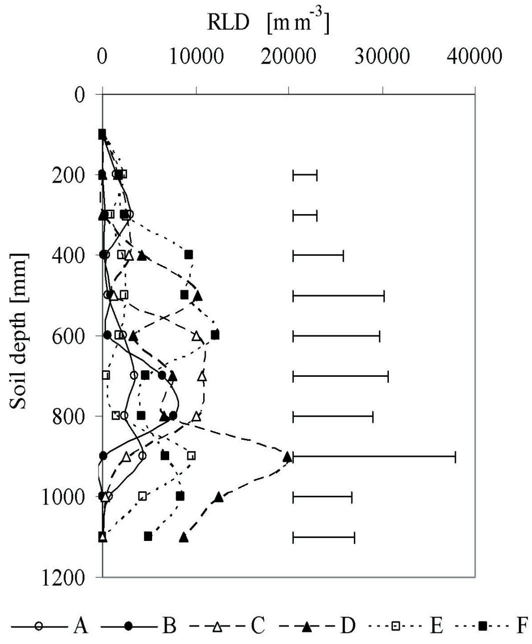

Fig. 4: Profiles of mean root length density (RLD) of the 6 vines (**A**-**F**). Error bars indicate $LSD_{95\%}$ for each depth with n = 6 (3 tubes x 2 sight-directions).

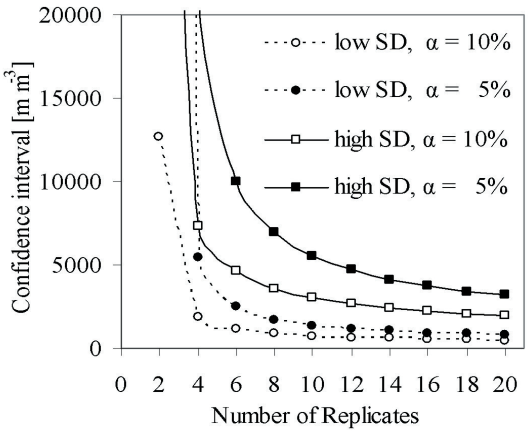

Fig. 5: Confidence interval as related from standard deviation (low SD = 1000 m·m^{-3}; high SD = 4000 m·m^{-3}), significance level α and repetition.

and RITCHIE (1983) recommend a minimum of 8 tubes for minirhizotron observation and according to TAYLOR *et al.* (1999) the same number is required for the soil core method to estimate RLD, but these recommendations were based on investigation on annual plants with higher RLD and lower variation.

On average, the cumulative root fraction followed a sigmoid curve (Fig. 6). According to the two maxima in RLD (Fig. 4), the cumulative root fraction of vine "A" and "E" followed a double sigmoid curve. The maximum of RLD was found in soil depths of 80 cm. Usually, vine roots are located between 20 and 40 cm (LOUBSER and MEYER 1986, MCLEAN *et al.* 1992, SOUTHEY 1992, REIMERS *et al.* 1994) and 40-60 cm under green cover (REIMERS *et al.* 1994). However, MCLEAN *et al.* (1992) reported the maximum of vine root number between 40 and 50 cm using minirhizotrons. Discrepancies with higher root ob-

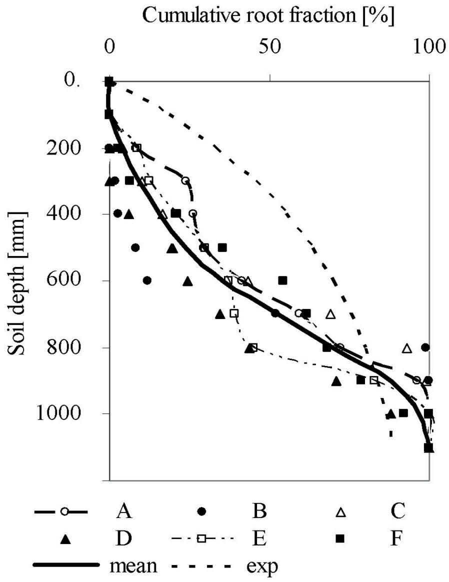

Fig. 6: Cumulative root distribution (in % of the total) of the 6 vines (**A-F**), of the mean and as an exponential function in soil depth 0-1200 mm. For each vine the values of the six minirhzotron observation transects have been combined to soil layers of 100 mm depth.

servations in deeper layers using minirhizotron instead of core sampling were reported for grain sorghum (UPCHURCH and RITCHIE 1983), too. SMART *et al.* (2006) reviewed *Vitis* root studies with profile wall methods. They found that growth distribution followed the exponential equation $Y = 1-\beta^d$ where Y is the cumulative fraction of roots, d is the soil depth in cm and β is a specific coefficient (with median 0.9826). For the rootstock 5 C they found 60-90 % of root distribution up to a soil depth of 60 cm. In this trial, it was only 35 % until 60 cm depth; furthermore, in this experiment cumulative root distribution followed a sigmoid function (Fig. 6). This is not surprising for a cumulative distribution function because the integral of any smooth, positive "bump-shaped" function will be sigmoid. On the other hand, it can be concluded that the exponential function for cumulative root distribution reported by SMART *et al.* (2006) underlies an RLD distribution with the maximum in the upper soil layer. Root observation is mostly not done on individual plants but in populations, so root-root interactions have to be considered. Competition between apple trees influences root distribution (ATKINSON 2000). In a Riesling/5 C vineyard at 1 km from the present trial, and under similar conditions using the monolith method, VIEHAUSER and ADAM (2005) found the highest RLD in soil depths of 70-85 cm when vines were spaced 2.4 x 2.0 m and a maximum in 130-145 cm when planted 0.6 x 2.0 m. When comparing root depth, plant density has to be considered.

M u l t i p l e a n a l y s i s o f t h e v a r i a n c e o f R L D : The standard deviation of RLD in soil depths to 600 mm was very high with a coefficient of variation of about 85 %. In the upper layer the coefficient of variation was about 60 %. Significant parts of this variance can be explained with differences between the plants, the

Table 1

Root length density (RLD) and statistic parameters as related to soil depth from 0-800 mm according to the minirhizotron observation in the field experiment (standard deviation (SD), portion of variance (PV) with the independent variables plant, angle, sight and their interactions

	0-200 mm	200-400 mm	400-600 mm	600-800 mm	0-800 mm
RLD [m m^{-3}]	600	2300	4500	5500	3200
SD [m m^{-3}]	500	1900	3800	3400	1700
PV [%]					
Plant (P)	6.9	17.4*	19.7*	12.3***	5.5***
Angle (A)	0.1	8.9*	9.8*	4.8*	2.1*
Sight (S)	0.1	0.9	1.2	0.5	0.2
P x A	28.2^{+}	21.4	46.0*	34.0***	13.5***
P x S	5.2	0.6	- [1)]	6.0*	0.8
A x S	0.5	0.2	5.7	0.0	0.2
P x A x S	2.1	4.2	- [1)]	6.7	2.8

+, *, **, ***: significant at α = 0.1, 0.05, 0.01, 0.001 % of root observation.
[1)] A singularity was encountered due to correlated dependents.

differently sloped minirhizotrons and their interaction (Tab. 1). As an example, Fig. 3 shows the RLD distribution of two vines for all minirhizotrons and observation sights. The observation direction had no significant effect on RLD. The angle of the tube installation influenced the measured RLD: on average the RLD observed in the 90° sloped minirhizotrons (2300 m m^{-3}) was significantly lower than for 60° (4100 m m^{-3}) and 45° (3400 m m^{-3}). BRAGG *et al.* (1983) recommended 60° sloped minirhizotrons rather than 90° because sloped tubes gave better estimates of root distribution than vertically installed tubes. DE RUIJTER *et al.* (1996), however, using the same data, concluded that vertical tubes showed a better correlation. One problem of minirhizotron observation is the danger of overestimating RLD at depth, which may be due to roots channelled down the vertical tube to soil interface. BRAGG *et al.* (1983) suggested sloped tubes so that root geotropism might encourage roots to grow away from the bottom side of the minirhizotrons. As in this experiment, the vertical (90°) tubes resulted in minor RLDs, an overestimation for V*itis* roots can not be excluded by sloped tube installation. The installation angle is especially important for plants with anisotropic root growth. Grass roots have a largely vertical growth habit, so they are more affected by the tube than tree roots which show a lateral distribution, because the vertical grass roots tend to follow the vertical minirhizotron tubes (BUCKLAND *et al.* 1993). The adventitious roots of the *Vitis* rootstock used here (Kober 5 BB und 5 C Geisenheim) grow in a wide angle with a rather lateral distribution, too (WEBER 1984); so vertically installed tubes do not influence root growth. Furthermore, in this experiment the angle of tube installation showed minor significance. The portion of variance due to different plants and especially due to the interaction between plant and angle was much higher. In other words, the angle for which higher RLD could be observed depended on the individual vine plant. For this, two different explanations are possible: First, the individual plants might have differed in their spatial root system which, in that case, was observed by different RLD due to the installation angle. Second, the difference of the installation angle between the vine plants might be due to the variation of root growth of the single plant, too. Therefore the interaction between plant and angle might only reflect the variation between the replicated minirhizotron. Replication effect and installation angle effect could not be separated in this trial as there was no replicated installation angle for one vine plant.

Minirhizotron vs. soil sampling: RLD in the pot experiment according to the monolith method and the estimated RLD according to minirhizotron did not correlate (Fig. 7). Direct soil sampling is often taken as the standard method for quantitative root measurements (Smit *et al.* 2000). But in this experiment considerable losses of roots smaller than 2 mm due to the washing procedure using the sieve could be realized. With the minirhizotron method this root fraction was counted, so this is also a source of discrepancy when comparing the methods. In contrast to this several researchers have found that root observation using minirhizotron is significantly related to root length resp. RLD assessed by other methods concluding that the minirhizotron technique will allow quantitative analysis of root growth: Box and Ramseur (1993) found minirhizotron counts of winter wheat roots related to RLD using the soil core method with a correlation coefficient (r) of 0.94. Bragg *et al.* (1983) reported a correlation coefficient between 0.8 and 0.9 for root observation with sloped minirhizotron compared to the core method on spring oat crop. Meyers and Barrs (1985) found r between 0.88 and 0.98 when investigating wheat root distribution. Vos and Groenwold (1983) investigated wheat and potatoes and reported correlation coefficients of r = 0.51 and 0.76 respectively. On the other hand there are well known discrepancies between the minirhizotron method and destructive sampling methods of estimating RLD like the underestimation in upper soil layers and the overestimation

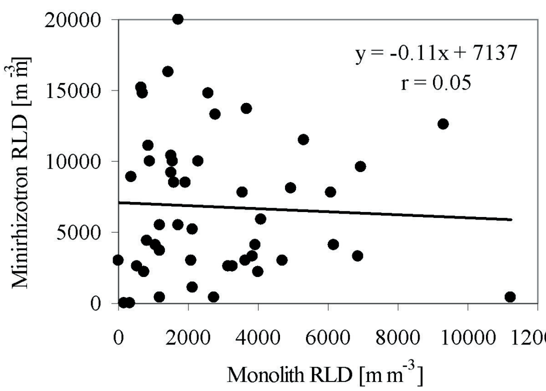

Fig. 7: Relation between root length density (RLD) according to the minirhizotron and the monolith method (r not significant at $\alpha = 0.05$) in the pot experiment.

at deeper soil depths mentioned above. Sometimes, good correlations were only found when data from the upper soil layer had been excluded (Upchurch and Ritchie 1983, Buckland *et al.* 1993). For grain sorghum, Upchurch and Ritchie (1983) reported very little correlation (r = 0.16) between the results of single tubes and bulk soil RLD according to the core method. Using means of 8 tubes r increased to 0.35, and if surface < 200 mm was eliminated r was 0.81. However, even researchers reporting significant relations between minirhizotron and soil core method often recommended thorough calibration for each set of experimental conditions like soil, climate, crop cultivar and minirhizotron installation technique (Vos and Groenwold 1983, Box and Ramseur 1993). It must still be considered that the high correlation coefficients have been found for annual plants with a more homogenous root distribution and with a higher RLD than vine. Nevertheless, even if the results were not encouraging, there is still a lack of comparing the minirhizotron method with soil sampling methods in field experiments. Root development with minirhizotron can be observed on the same vines at the same place at different times. So the minirhizotron method is a very useful instrument to investigate the dynamics of root growth (Smit *et al.* 2000, Lehnart *et al.* 2008). A combination of (core) soil sampling and the minirhizotron method could profit from the benefits of both methods.

Conclusion

There are a lot of methods of measuring roots, none of the methods is applicable for all situations (Böhm 1979). When chronological changes in root development have to be observed the minirhizotron method is one of the best methods. For the investigation of the spatial distribution at one moment in time direct sampling methods are preferred. The monolith or the profile wall method have the further advantage that information about soil conditions can be gathered to explain root distribution (Böhm 1979, Atkinson 2000). However it would be useful to derive spatial root distribution from the minirhizotron observations. This trial showed that the quantitative RLD measurement of *Vitis* roots using minirhizotron is difficult. Due to high variation it is necessary to investigate several individual plants with replicate tubes. As a compromise between statistical requiries and the work load at least 4 plants with 14 tubes as a total should be used. RLD distribution as a function of the installation angle differed due to the individual vine plants. Taking this into consideration, differently sloped tubes could even minimise the influence of root anisotropy on the estimation of RLD. Further measurements should be compared under field conditions e.g. with the soil core or the monolith method.

Acknowledgements

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