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Research Article

APPLICATION OF THE MINIRHIZOTRON TECHNIQUE TO STUDYING THE ROOTS OF FRUIT PLANTS

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ABSTRACT

Minirhizotron, a non-destructive technique is based on the application of transparent tubes, located in plant's root zone. This method has been known since the beginning of 20th century and is used for plant root's observations, especially in forest trees (Scots pine, Norway spruce, silver fir, birch), steppe grasses, vegetables and cereals. Minirhizotron technique is also applicable to pomological plants observations, mostly apples, but many others orchard species were observed with this method last years. The study of root growth dynamics in fruit plants using the non-destructive, minirhizotron method is conducted in the Pomological Orchard in Skierniewice. The objects of the observations are the roots of: apple trees cultivar. 'Gold Milenium', blackcurrant bushes cultivar 'Tiben' and sweet cherry cultivar 'Vanda'. The observations were carried out monthly over a period of from March to November.

Key words: minirhizotron, fine roots, pomological plants.

INTRODUCTION

A significant role in the functioning of the root system of plants is played by fine roots assumed to be the roots that are less than 1-2 mm in diameter [9, 68, 72]. In some publications, fine roots are defined as having a diameter of less than 0.5 mm [30] or much more than 2mm [10]. Fine roots play a significant role in water and nutrients uptake, the release of root exudates, the exchange of minerals and organic compounds between plants and rhizosphere and mycorrhizas formation. Water uptake by the roots is also an essential aspect of a plant's existence . In many studies aiming at creating a water uptake model, the length of the root system is used as its basis [14, 23, 57, 61]. Some researchers claim that roots of different age and in various locations are anatomically and physiologically diversified [11, 35, 44, 47, 48, 49]. Fine roots water uptake potential in soil volume unit varies according to their number, length, surface area and volume, number and length of root hairs [17, 18, 21, 53, 54, 55]. Fine roots also take part in the storage of organic matter and nutrients. Their lifespan has a significant effect on plant growth and fruit yield, on the carbon cycle and circulation of other nutrients in ecosystems, as well as on the competitiveness between different species of soil-inhabiting organisms [11]. Fine root turnover supplies the soil

with four to five times more organic carbon than the decomposing remains of the above-ground parts of plants, leaves in particular [37]. Parasitic microorganisms in the rhizosphere contribute to the release of mineral and organic components from fine roots to the soil, which are then used by saprotrophic organisms. Fine roots, thanks to their high metabolic activity, have also a significant effect on the processes taking place in the rhizosphere and the organisms living there, particularly bacteria and fungi, among which an essential role is played by mycorrhizal fungi [21].

ROOTS AND ROOT ZONE OBSERVATIONS USING MINIRHIZOTRONS

Minirhizotrons (defined here as transparent tubes placed in soil) and specialist programs for root images analysis both have proved to be very useful tools for studying *in situ* the phenomena taking place in the soil, particularly for observing and examining the growth of plant roots and the processes, occurring in their vicinity. Such devices are used not only to study the dynamics of the growth and development of fine roots, but also to survey other soil organisms e.g. mycorrhizal fungi (hyphae and spores) or small invertebrates, to determine the rate of organic matter decomposition. Minirhizotrons used in various conditions, either field or controlled environment allow validating the results of many laboratory experiments [8, 46] or field observations based on destructive methods e.g. soil profiles [58]. Minirhizotrons are particularly useful for studying processes that take place simultaneously, for example, the production, dying-out and disappearance of roots, which cannot be scrutinized by means of conventional tools, such as ingrowth cores and soil cylinders for studying roots, the use of which has a destructive effect on the root system, making such studies impossible [32, 33, 41, 52]. However, these methods should constitute an essential supplement to the research based on the use of minirhizotrons as they make it possible to determine the properties of the rhizosphere, such as dry root weight, root composition, and the presence of mycorrhizal fungi and rhizosphere bacteria [43, 45, 60].

Important objects of root and rhizosphere studies are mycorrhizas, which form a symbiosis between plant roots and mycorrhizal fungi. One such example are ectomycorrhizas, which manifest themselves on the surface of roots as mouldy growths and characteristically forked roots, quite transparent in photographs of the root systems of coniferous trees [63]. Minirhizotrons are also used for studying arbuscular mycorrhizas under controlled conditions [24, 31] and in the field [50, 64]. The devices in question were also applied by Kosola et al. [34] in their studies of the response of *Citrus volkameriana* plants to the infection with the pathogenic fungus *Phytophthora nicotianae*. Eizenberg et al. [22] used minirhizotrons for observation of sunflower roots colonization by broomrape (*Orobanche cumana* Wallr.) and for examining the influence of soil applied herbicide on this parasite growth. Minirhizotrons are useful for observations of the effects of various plant protection and fertilization techniques impacting the growth dynamics of fine roots [13, 59, 70].

Minirhizotron technique has many limitations and difficulties appearing at the level of both image capturing time and image analysis phase. In image capture time one of the most important factors are weather conditions, because the devices used (camera and computer) are susceptible to water. The temperature is an important factor, because battery supplied devices are temperature sensitive (in very high and low temperatures their efficiency falls dramatically).Low temperature influences minirhizotrons, especially with winter soil freezing, causing soil and tubes movements. Tube movements make difficulties in subsequent observations, wherever soil movements cover some roots what makes them invisible. Dewdrops on tubes' external walls also make roots invisible. Other technical problems worth mentioning are caused by insects (especially ants) which may colonize tubes (author's observations). The above problems render difficulties in image analysis phase: problems with distinguishing between alive and dead roots or between young tree roots and roots of some dicotyledonous weeds.

OBSERVATION OF POMOLOGICAL PLANTS IN THE WORLD

Studies of fruit plants by means of minirhizotrons are not as common as the studies of plants in forest and meadow ecosystems [15, 29, 30, 63] or of cereals and vegetables in field [27, 36, 42, 43]. However, this technique has been already commonly used to examine such fruit plant species as: apple [20, 67], peach [2, 5, 69, 70], grapevine [3,

6, 7, 16, 30, 38, 40, 56], pear [51], sour cherry [4] grapefruit [11], orange [12], blackcurrant [39], olive trees [66] and some others [8, 35, 65].

The main fruit crop which is examined with minithizotron technique is apple (*Malus domestica* Borkh.) (Figure 1). Wells and Eissenstat [67] examined the influence of apple tree fine roots diameter on their overwinter survivorship in 1994/1995 and 1995/1996 winters. They observed that roots under 0.3 mm in diameter had 3 to 12% survivors, in class 0.3–0.5 mm had 30% and in class 0.5–1.1 mm had` 55–60% of survived roots. Smaller roots (<0.3 mm) have median lifetime under two months, while roots in class 0.3–0.5 mm lived 97 day in 1994/1995 and 143 days in 1995/1996. The biggest fine roots had median survival lifetimes 211 days or more. Roots neighborhood was also shown as an important factor of fine roots survival. Fine roots of larger diameters existed alone or with single neighbors, smaller roots were likely to have frequent number of neighboring roots and relating higher risk of mortality. Eissenstat et al. [19] examined fine roots growth of two apple cultivars: Gala on M9 rootstock and Golden Delicious on M9 rootstock. They observed double root flush: one around full bloom and the second in midsummer or after the harvest of fruits in 2002, but in 2003 no evidence of spring root flush was seen.

Characterization of double apple root flush is confirmation of previous Abod and Webster's [1] observations. First flush occurred in spring before buds breaking, the second one occurred late summer or early autumn after shoots growth termination. Between June and August only few new roots are observed. Eissenstat et al. [20] observed, that the majority of apple 'Red Chief Delicious' fine roots (80% to 85%) occurred in late spring and autumn did not survive winter and those roots which survived got brownish color. As reported in the same article, 45% newly formed roots of watered trees survived the period between June and September, while in no watered trees only 23% of newly formed roots survived. Late summer root growth flush was observed after fruit harvesting.

Yao et al. [71] used minirhizotrons for observation of three apple rootstocks clones growth planted in soil with ARD (Apple Replant Disease). After removing old trees, new plants were placed in old tree rows or in old grass lanes. There were three soil treatments (fumigation, compost amendment and untreated checks). Preplant soil treatments and planting position had no apparent influence on root growth, but rootstock clones influenced root growth. Trees on CG.6219 rootstock were bigger and gave higher yield than the trees on G.30 and M.7 rootstock. Roots of CG.6210 had more roots in deeper soil levels and lived up to two times longer than roots of other examined rootstocks.

Anderson et al. [3], based on minirhizotron observations, observed that on the grapevine 'Concord' roots lifespan was influenced by such condition as root depth under soil surface, diameter and phenological phase of root appearing. Roots located in deeper soil layers lived longer than the roots immediately under soil surface; 10-20% longer for roots growing on depth 40 cm comparing to roots growing 10 cm under soil surface. Roots formed in the period of 30 days

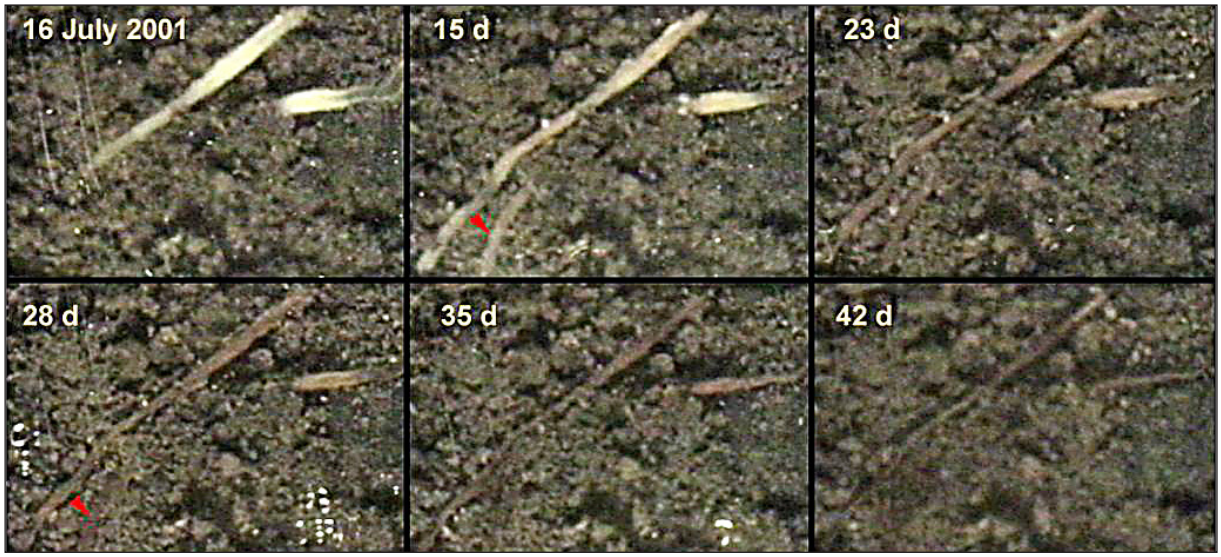

Fig. 1. Apple fine roots chronosequence. Author D. Eissenstat (with author's approval)

after blooming or 30 days before fruit ripening lived longer than roots occurred near bloom period. Roots with diameter above 0,4 mm lived more than 35% longer than smaller roots. Canopy pruning and irrigation effects seemed to vary with growing-season environmental conditions.

Bauerle et al. [7] observed roots lifespan and morphology in wet and dry soil for the grapevine 'Merlot' on the high shoot growth promoting rootstock 1103 Paulsen and on the low shoot growth promoting rootstock 101-14 Millardet de Gramanet in California vineyard. The 1103P rootstock produced generally more roots in irrigated soil, in comparison to 101-14MDG. In dry conditions lateral fine roots of 1103P in 20 cm top soil layer had 30% lower diameter than in irrigated soils and were also thinner than in deeper soil layer. This rootstock root morphology was very dependent on soil water content. The second rootstock exhibited little variance in root diameter under irrigated and unirrigated conditions. In soil layer 20–60 cm both rootstock root diameters were generally similar for irrigated and unirrigated soils. Fine roots lifetime depends on soil water content. In dry stress lifetime was similar for both rootstock but in summer, in irrigated conditions roots of 101-14MDG had longer median lifespan (95 days) than 1103P rootstock (67 days).

Lehnart et al. [38] examined the 'Riesling' grapevine root lifespan and distribution in soil profile with minirhizotrons. They described three groups of root length density (RLD), each group localized in different soil depth. The first group, with maximum RLD was localized on 40–60 cm and 80–100 cm. The second one was on the depth 60–80 cm. The third group, with smaller RLD, had three times less roots than other groups, and their highest number was at 80–100 cm. Authors also observed two peaks of intensive roots growth, depending on year and grapevine plant. The first period was correlated with shoots growth stop and the second appeared after harvesting.

Basile et al. [5] worked with peach trees. They researched the influence of size controlling and invigorating peach rootstocks on morphology and growth patterns of fine roots. They use five rootstocks: a vigorous control (Nemaguard), three intermediate vigor rootstocks (Hiawatha, K119-50 and P30-135) and a semi-dwarf rootstock (K146-43). They showed that almond origin rootstock K119-50 (in genetic background) produced significantly more new roots per year and had the greatest quantity of roots than other rootstocks. The semi-dwarf rootstock had thicker roots than the other rootstocks. The greatest number of roots occurred during spring and decreased during the following periods. The second period of intensive fine roots growth for all rootstocks occurred after fruit harvest. They showed also that the 'Nemaguard' produced more fine roots in soil layer between 17–26 cm, whereas fine roots of K146-43 and K119-50 were produced in soil layer 69–78 cm (Figure 2).

The Abrisqueta et al. [2] examined irrigation regimes in two young peach trees: continuous deficit irrigation and partial rootzone drying (PRD) on the root dynamics. Testing irrigation regimes was important because of water deficiencies in south Spain. They showed that deficit irrigation reduces soil water content and causes considerable reduction in root growth. Trees with continuous deficit irrigation treatment had 73% lower root length density than the control trees. A partial rootzone drying treatment increases root length density in

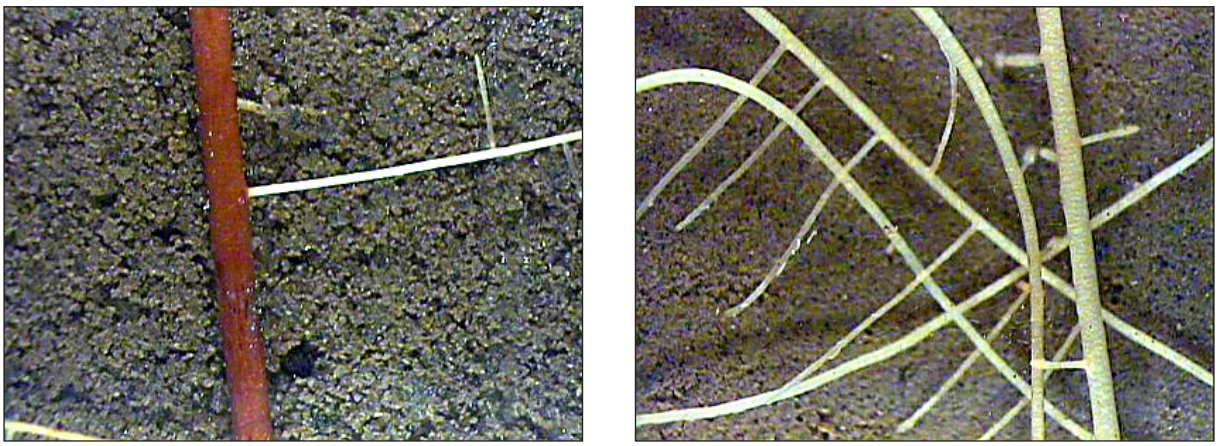

Fig. 2. Example roots of peach rootstock (without cv. identification) in experiments made by B. Basile. Images taken using minirhizotron camera (with author's approval)

comparison to continuous deficit but the values were lower than the ones obtained in control group (42% lower), but the water usage in these deficit regimes was similar. They also observed higher root growth after fruit harvest in all combinations.

Wells et al. [70] used minirhizotrons to observe the soil insects suppression on the demography of peach fine roots over two growing seasons (1996 and 1997). They also examined the influence of insecticides on the insects' prey on the fine roots. The use of broad spectrum insecticide (Lorsban 4E with active ingredient chlorpyrifos) reduced insects' roots feeding and decreased the risk of fine roots mortality and extends the median lifespan of these roots (from 45 to 145 days in comparison to the untreated control trees). Smaller roots were more frequently eaten than larger diameter roots. Application of pesticide also delayed the development of roots' brown pigmentation and had no effect on nitrogen and phosphorus content in soil.

Tanner et al. [59] observed, with minirhizotrons, that roots of peach had longer median lifespan, in comparison to the untreated control, when grown in solarized (thermal sterilized) soil (27 days) or methyl bromide fumigated soil (28 days). Soil solarization also gave higher percentage of roots that underwent secondary development than other treatments. Trees planted in solarized or fumigated soil had also bigger trunk diameter than the trees planted in untreated soil.

Weisbein et al. [66] studied the influence of water salinity on the vegetative and reproductive response of the olive (*Olea europaea*). They used two water regimes (tap water and moderate saline water) and they showed that the saline water minifestes low rate of retardation in comparison to tap water. The obtained results suggest that olive trees cultivation in semiarid conditions is closely related to the proper conditions (Figure 3).

Not only are fruit trees examined with minithizotrons. Lindhard Pedersem [39] used minirhizotrons for observation roots of blackcurrant bushes cultivars 'Titania', 'Ben Lomond', 'Farleigh' and 'Intercontinental' grown with "green mulch" plants. Majority of roots were produced at 75 up to 125 cm above soil surface. Cultivar 'Titania' had roots deeper than 'Ben Lomond'. Bushes grown with "green mulch" plants had bigger root system than plant without cover.

Valenzuela-Estrada et al. [65] used minirhizotrons to measure roots lifespan of blueberry cultivar 'Blue Crop'. They observed that live time of finest roots (1st and 2nd order) was about 4 months; the thicker roots (3rd order) median lifespan was 4 up to 5 months.

Minirhizotrons were also used for explanation why cranberry cv. 'Ben Lear' gave smaller yield in comparison to cv. Stevens', when they grow in field [8]. Authors showed that poor yielding of 'Ben Lear' in reduced irrigation conditions is caused by shallow and smaller root system, high relation of leaf surface area to root length and poor assimilates transfer from leaves to roots.

MINIRHIZOTRON OBSERVATIONS AT THE RIH'S POMOLOGICAL ORCHARD

At the Research Institute of Horticulture (RIH), the technique of observing root systems with minirhizotrons was introduced in 2007 for

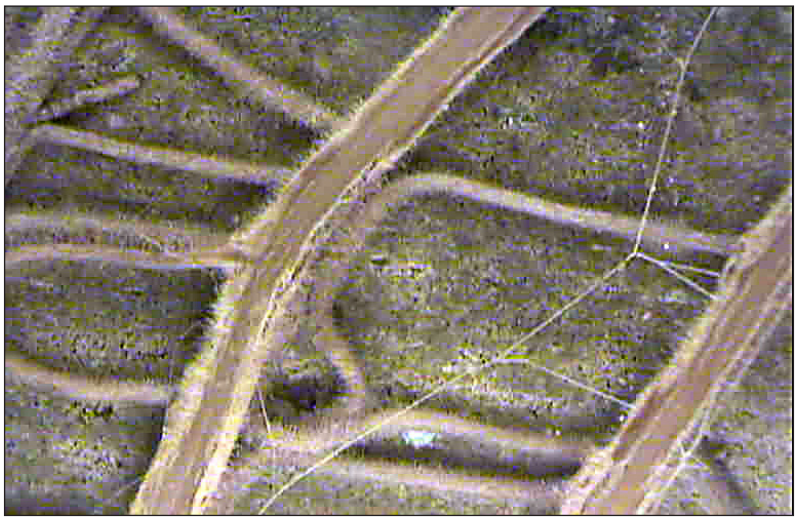

Fig. 3. Olive tree cv. Barnea images. Author J. Ephrath (with author approval)

the purpose of studying root growth dynamics in apple cultivar 'Gold Milenium' and blackcurrant cultivar 'Tiben' grown in the Pomological Orchard of the Institute in Skierniewice [N 51°57'37'', E 20°09'40'']. The apple trees and black currant bushes under study are also the subject of an experiment on the effect of various organic mulches on vegetative growth and fruit yield. From 2011 there is also additional object –sweet cherry trees cultivar 'Vanda'. Sweet cherry is the object of experiment with application of organic origin fertilizers (biofertilizers) and their effect on vegetative growth and yielding. Furthermore, from 2010, in cooperation with University of Life Sciences in Warsaw, four sour cherry cultivars: 'Sabina', 'Koral', 'Łutówka' i 'Debreceni Botermo', grafted on 'Mahaleb' rootstock are examined.

Preliminary results of image analyses indicate that the number and lifespan of the roots forming depend more on plant species than the applied mulches. A greater number of roots is formed by blackcurrant bushes than by apple trees. It has been observed, however, that within the species there are differences in the dynamics of the development of the root systems depending on the mulch used as compared to NPK fertilization. In comparison to NPK fertilization, organic mulches have been found to increase the incidence and biodiversity of beneficial mesofauna in the root zone.

Preliminary results of black currant bushes root image analyses showed that the species and cultivar had bigger effect on root growth dynamic than the kind of soil treatment [25] (specific data in preparation). Mulching also affects root growth. NPK fertilizer treated plants produce fewer roots than plants treated with organic mulches. Organic mulches increased also the number of observed mesofauna in root zone. Application of biofertilizers to sweet cherry trees also gave positive effect on roots number and growth.

The second part of the study concerns the assessment of the effects of using bioproducts (Bioilsa, BioFeed Ecomix, Ausma) or bacterial-mycorrhizal inoculum on the dynamics of the development of roots of sweet cherry cultivar 'Vanda'. The bioproducts used have been found to affect the lifespan of roots and the number of newly formed roots. The control in both experiments is fertilization with NPK.

In sour cherry research it is observed, that the longest lifespan had cultivar 'Sabina' and the shortest cultivar 'Łutówka'. The results obtained show that the rootstock influenced root dynamics in soil [26]. Besides,there is possibility that the grafted cultivar also have influence on roots' development. The roots of the cultivar 'Sabina' were found to have the longest average lifespan, while those of 'Łutówka' the shortest. The results obtained in this experiment indicate a significant effect of the rootstock on the development of the root system of the sour cherry cultivars under study. Furthermore, it is also possible that the grafted cultivar, not only the rootstock, can affect the root development and the lifespan of the studied fruit crop species.

The obtained results will be used to determine the effects of different methods of cultivation, i.e. mulching, mycorrhization and biofertilizer applications, on the growth and lifespan of roots in fruit plants. The next paper on the extended work on this topic is under preparation.

CONCLUSIONS

1. The minirhizotron technique is a suitable method of studying the growth and development dynamics of orchard plant roots in field conditions and is widely used in plants' life processes examination.
2. This method allows also observation of plant roots in controlled conditions, both in soil and in pots.
3. Apart from the possibility of observing root growth and development this technique allows detection and identification of other soil organisms such as arthropods, nematodes, annelids, and also saprotrophic or mycorrhizal fungi mycelia.
4. The used minirhizotron technique has been optimized (tubes location in soil and image capture time) for the species studied. However, further studies will be carried out to determine the effects of agrotechnical and varietal factors on root growth dynamics in apple and blackcurrant.

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