Tree Physiology 27, 155–160

Polyphenols in the woody roots of Norway spruce and European beech reduce TTC

ANIKA K. RICHTER,[1,3] EMMANUEL FROSSARD[2] and IVANO BRUNNER[1]

[1] *Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Zuercherstr. 111, CH-8903 Birmensdorf, Switzerland*

[2] *Institute of Plant Science, Eschikon 33, CH-8315 Lindau, Switzerland*

[3] *Corresponding author (anika.richter@wsl.ch)*

Received January 26, 2006; accepted March 17, 2006; published online October 2, 2006

Summary A common method to determine the vitality of fine root tissue is the measurement of respiratory activity with triphenyltetrazolium chloride (TTC). The colorless TTC is reduced to the red-colored triphenyl formazan (TF) as a result of the dehydrogenase activity of the mitochondrial respiratory chain. However, measurements with woody fine roots of adult Norway spruce and European beech trees showed that dead control roots had a high potential to react with TTC. High reactivity was found in boiled fine roots and the bark of coarse roots, but not in the boiled wood of coarse roots. By sequential extraction of dried and ground adult Norway spruce fine roots, reactivity with TTC was reduced by about 75% (water extraction), 93% (water/methanol extraction) and 94% (water/acetone extraction). The water extract reacted with TTC in the same way as polyphenols such as lignin, catechin and epicatechin. Boiling did not affect the extent to which fine roots of adult trees reduced TTC, whereas it greatly reduced TTC reduction by seedling roots. Application of the TTC test to roots of spruce seedlings subjected to increasing drought showed a progressive decrease in TTC reduction. The decrease in TTC reduction was paralleled by a reduction in O_2 consumption, thus supporting the conclusion that for roots with a low polyphenol content the TTC test provides a valid assessment of tissue vitality. Our results suggest, however, that the TTC test should not be applied to the fine roots of adult trees because of their high content of polyphenolic compounds whose reaction with TTC masks changes in TTC reduction due to changes in the respiratory capacity of the tissue.

Keywords: Fagus sylvatica, fine root vitality, Picea abies, respiration, tannins, tetrazolium chloride.

Introduction

The vitality of fine roots is commonly taken to reflect the effects of such factors as frost, drought or soil acidity on forest tree health (Clemensson-Lindell and Persson 1995, Bakker 1999, Stattin and Lindström 1999, Zhu et al. 2002, Vanguelova et al. 2005). For example, acidic deposition has caused acidification of many forest soils (Blaser et al. 1999, Graf Pannatier et al. 2005), which may have affected fine root activity, turnover (Godbold et al. 2003, Vanguelova et al. 2005) and growth (Jentschke et al. 2001). However, methods for assessing the physiological state of the fine roots of forest trees have been little investigated.

One way of assessing the condition of fine roots is by their color and brittleness (Helmisaari and Hallbäcken 1999, Comas et al. 2000). However, this method allows only the distinction between living and dead roots. Another approach is to measure root morphological parameters, such as specific root length (cm g_{DM}^{-1}) and specific root tip density (root tips g_{DM}^{-1}) (Clemensson-Lindell and Persson 1995, Godbold et al. 2003). These parameters are often used to evaluate the reactions of fine roots to different soil conditions or nutrient supplies (Hodge 2004), but do not reflect physiological vitality.

A commonly used physiological measure of tissue vitality is the triphenyltetrazolium chloride (TTC) test. Colorless TTC accepts electrons from the electron transport chain of mitochondria reducing it to the red-colored triphenyl formazan (TF) (Clemensson-Lindell 1994, Comas et al. 2000, Ruf and Brunner 2003). Under favorable conditions, the reaction is directly linked to the rate of respiration. However, our tests with boiled (dead) tree roots revealed that not only the respiratory chain, but also some reactive substances within the root tissues, can reduce TTC. These substances prevent accurate measurement of respiratory activity by the TTC reaction. The aim of the present study was to investigate: (1) in which part of the woody tree roots TTC-reactive substances occur; (2) which substances in the fine roots of the two main tree species of the Swiss Plateau, Norway spruce (*Picea abies* (L.) Karst.) and European beech (*Fagus sylvatica* L.), react with TTC; (3) whether the fine roots of adult trees react to TTC in the same way as roots of tree seedlings; and (4) whether the TTC test correlates with O_2 consumption.

Material and methods

Fine root sampling and preparation

Roots of Norway spruce and European beech were sampled at a forest site at Vordemwald (634/236), Switzerland (for de-

tails, see Brunner et al. 2004). Soil cores including fine roots were taken with a soil corer (5 cm diameter) in the Ah-horizon (0–5 cm) 1 m from the tree stems. The samples were immediately wrapped in plastic bags to prevent water loss and stored at 1 °C in the laboratory for no longer than 1 week.

The soil cores were sieved and the roots collected and gently washed with tap water. The roots were assessed visually. Roots with flexible cell walls, a white stele and turgid and unbroken tips were classified as alive (Hertel and Leuschner 2002). Living roots were divided into fine roots (< 2 cm) and coarse (2–5 cm) roots and stored in water at 0 °C for no longer than 1 h.

Living fine roots were cut into segments 1–2 mm long, mixed and divided into three parts. One part was immediately used for the TTC test (see below). The other parts served as controls. For one control, 100 mg fresh mass was boiled in 150 µl of distilled water for 20 min in 2-ml Eppendorf tubes. For the other control, the roots were dried for 48 h at 60 °C and ground for 5 min at 90% speed in a swing mill (MM 2000; Retsch, Haan, Germany).

For tissue-specific measurements, the bark and wood of the coarse roots were separated and cut into segments 1–2 mm long. These tissues were also divided into three parts and treated in the same way as the three fractions of fine roots.

TTC test

To prepare the samples, 100 mg fresh tissue, 100 mg fresh tissue boiled or 23 mg of dried and ground tissue (the dry mass of 100 mg fresh fine roots) was transferred to 2-ml Eppendorf tubes containing 1.5 ml of TTC buffer (0.1 M potassium phosphate buffer (pH 7.0) with 0.6% TTC and 0.05% Tween 20; according to Ruf and Brunner (2003)). The samples were placed under reduced pressure (300 hPa) for 15 min to remove air from the intercellular spaces and to support the infiltration of the TTC buffer. The samples were incubated for 20 h at 30 °C in darkness. During incubation, TTC was reduced to TF. After incubation, the TTC buffer was decanted and the root pieces were washed twice with 2 ml of distilled water. Two steel balls (2 mm diameter) were added to each sample and the root pieces were milled for 5 min at 90% speed in the swing mill. In the case of the dried samples, the TTC buffer was decanted after centrifugation for 15 min at 10,000 *g*.

To extract the alcohol-soluble TF from each sample, 1 ml of 96% alcohol was added and the samples vortexed for 10 s. The samples were centrifuged for 2 min at 10,000 *g* and the absorption of 300 µl of the alcohol extract containing dissolved TF was measured photometrically at 520 nm with a microplate reader (VersaMax; Molecular Devices, Sunnyvale, CA). Samples were dried for 48 h at 60 °C and weighed. The reactivity of the samples with TTC was measured as absorption of TF per g dry mass ($A_{520}\ g_{DM}^{-1}$).

Extractions of fine roots and the bark of coarse roots

Roots were extracted to investigate the group of substances that react with TTC in the control samples. Samples of 5 g of dried and ground Norway spruce and European beech fine roots and coarse root bark were extracted for 2 h under continuous shaking with 160 ml of a 1:1 (v/v) water:methanol mixture (Matthews et al. 1997). The extraction residue was filtered from the root powder (Filter No. 589, Schleicher and Schuell AG, Feldmeilen) under reduced pressure (300 hPa). The extraction residue was dried for 48 h at 60 °C, and 23 mg of each residue was tested for their reactivity with TTC (see above).

Sequential extraction of the fine roots

Fine roots of adult Norway spruce were sequentially extracted as described by Peng et al. (1991) and Matthews et al. (1997). First, 6 g of dried and ground fine roots was extracted with 240 ml of distilled water, filtered and dried. The extract was lyophilized and stored at 1 °C. Second, 4-g aliquots of the water extraction residue were further extracted with 160 ml of a water:methanol mixture (1:1; v/v), filtered and dried. Finally, 3 g of the water:methanol extraction residue were extracted with 120 ml of water:acetone mixture (3:7; v/v), filtered and dried. The dried fine root material and the extraction residues (each 23 mg) were tested for reactivity with TTC.

Reactivity of extract and cell components

To compare the reactivity of the lyophilized water extract with the fine root powder of Norway spruce and other common cell constituents of roots, samples were tested for their reactivity with TTC. Other constituents included: cellulose (for column chromatography (Macherey, Nagel and Co., Düren, Germany); pectin (from apples; Fluka, Buchs, Switzerland); lignin (from fir wood (*Pseudotsuga menziesii* (Mirbel) Franco), Bender and Hobein AG, Zürich); tannic acid (from oaks, Fluka); ellagic acid (Fluka); epicatechin (Fluka); and catechin (Fluka). As controls, the assay was run with fine quartz sand (washed; Merck, Darmstadt, Germany) and with water. One mg of each of these substances was added to 1.5 ml of TTC buffer in reaction tubes and their reactivity observed.

Effect of the age of tree roots on the TTC reduction

Living fine roots of different ages were collected from the forest site in Vordemwald. Fine roots from adult Norway spruce and European beech trees, which were assumed to be older than six months, were sampled in soil cores. Seedlings of beech and spruce (1–6 months), which germinated in the same forest site, were excavated and taken with an intact root ball to the laboratory. In addition, seeds (Tägerwilen, #845, seed collection WSL) of Norway spruce were sown in pots containing forest soil of the Ah-horizon from Vordemwald. The seedlings were grown for either one or two months in the greenhouse. For vitality measurements with TTC, fine roots of adult trees were treated as described above. For the measurements of seedling roots, the soil was removed and the fine roots were gently washed with tap water. Boiled roots from each age class served as controls (see above). The fine roots (alive or boiled) were tested for reactivity with TTC.

Effect of drought on the fine root vitality

To investigate the effect of drought on seedling root vitality under different soil conditions, samples were collected from the Ah-horizon (base saturation (BS) 6.5%, carbon concentra-

tion 7.9%) and from the B-horizon (BS 3.1%, carbon concentration 1.0%). Soil monoliths were taken with a HUMAX soil corer (5 cm diameter; Lucerne, Switzerland) in plastic tubes. Monoliths were taken from six sampling sites 2 m apart with three replicates each.

In the laboratory, the plastic tubes containing the soil monoliths were divided into the Ah- (0–8 cm) and the B- (8–16 cm) horizons. Each horizon was sown with 20 seeds of Norway spruce (Tägerwilen, #845, seed collection WSL) and placed in the greenhouse. The seedlings were watered three times weekly with about 10 ml of distilled water. After six weeks, watering was stopped. Reactivity with TTC and O_2 consumption of the seedlings in each sample was tested 1, 4, 8, 15 and 22 day(s) after the watering ended.

Soil water losses were calculated by subtracting the dry mass (after 4 days of drying at 50 °C) from the fresh mass. Samples without watering for 18 days were dried for 4 days at 50 °C and served as controls. For the TTC-measurements, 100 mg fresh seedling roots of one sample (three replicates) of each horizon were used (see above). For the consumption of O_2 measurement, seedling roots were measured for 20 min with a Clark-type O_2 electrode (Hansatech, King's Lynn, U.K.). For this measurement, 25–100 mg fresh roots were placed in 2.5 ml of aerated 1 mM $CaSO_4$ + 5 mM MES buffer (adjusted with KOH to pH 5.5; Comas and Eissenstat 2004). The temperature of the whole system was kept constant at 25 °C. Roots were then dried for 48 h at 60 °C and weighed. Respiration was expressed as O_2 consumption per g dry mass (nmol O_2 g^{-1} s^{-1}).

Statistical analyses

Three replicates were measured for each test. The data were subjected to two-way analysis of variance (ANOVA). The significance level was $P < 0.05$ by Fisher's PLSD test. All tests were calculated with StatView 5.0 (SAS Institute, Cary, NC).

Results

Reactivity with TTC

The TTC reactivity of living and dead fine roots, and of the bark and wood of coarse roots of both Norway spruce and European beech is summarized in Table 1. Except for the wood of European beech, the reactivity of the living samples was relatively high. There were almost no significant decreases in reactivity when the fine roots and bark were dried and (except in the case of bark) boiled. In contrast, the reactivity of the dead wood of coarse roots significantly decreased. The reactivity of the extraction residues with TTC, however, decreased significantly after a water/methanol extraction (79 to 87% (fine roots) and 92 to 97% (bark); Table 1).

When dried and ground Norway spruce fine root samples were sequentially extracted, a stepwise decrease in TTC reactivity was observed (Figure 1). After water extraction and the water:methanol extraction, the reactivity of the dried extraction residue decreased 75 and 93%, respectively. The last extraction (water:acetone) had no effect on TTC reactivity.

A comparison of the TTC reactivity of different cell-wall substances, dried and ground fine roots, the lyophilized water extract and controls revealed that some cell-wall constituents, such as cellulose and pectin, had almost no reactivity with TTC (Table 2), whereas lignin and substances belonging to the group of water-soluble polyphenols reacted strongly with TTC. In particular, the reactivities of epicatechin and catechin were high, as were those of the dried and ground fine roots and its water extract (Table 2). Almost no reactivity was recorded with the control substances (Table 2).

Effect of the ages of tree roots on TTC reduction

Living fine roots of seedlings of Norway spruce and European beech were two to three times more reactive with TTC than the living fine roots of the adult trees (Table 3). Boiling reduced the reactivity of seedling roots to about 11–13%, whereas boiling had little effect on the reactivity of fine roots of adult trees (80%).

Effect of drought on fine root vitality

Significant water losses in the soil monoliths of the two horizons were observed 22 days after watering was stopped (Figure 2a). The decrease in soil monolith water content was highly significant for both the time period and the horizon.

Root vitality measurements based on the reduction of TTC and the consumption of O_2 (Figures 2b and 2c) showed a slight increase in the vitality of the roots from Days 1–4 of drought. This increase was significant only in the case of O_2 consumption. Four days after watering stopped, fine root TTC reactiv-

Table 1. Triphenyltetrazolium chloride reactivity (absorbance of triphenyl formazan (TF) (Abs_{520} g_{DM}^{-1}) of living and dead root tissues and of the extraction residues of dead root tissues after a water:methanol (1:1; v/v) extraction. Different letters indicate significant differences ($P < 0.05$; n.d. = not determined).

Root tissues	Norway spruce			European beech		
	Fine roots	Coarse roots		Fine roots	Coarse roots	
	Total	Bark	Wood	Total	Bark	Wood
Living	196 a	108 b	128 a	191 a	266 a	54 a
Dead (boiled)	156 a	48 c	28 b	120 b	41 b	4 b
Dead (dried/ground)	203 a	252 a	9 b	140 ab	241 a	20 b
Residues after extraction	27 b	7 d	n.d.	30 c	6 b	n.d.

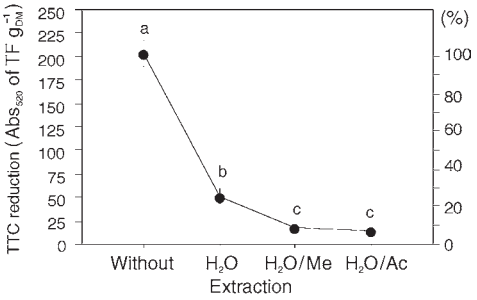

Figure 1. Mean reduction of the triphenyltetrazolium chloride (± SE) of dried and ground Norway spruce fine roots and their dried extraction residues after different extraction steps. Abbreviations: Me = methanol; and Ac = acetone. Different letters indicate a significant difference ($P < 0.05$).

ity decreased continuously and significantly and reached minimum values after 22 days of drought. There was no difference in the TTC reactivity with soil type.

Analysis of the relationship between seedling root TTC reactivity and the soil water content revealed a highly significant correlation (Figure 3a). Figure 3b shows that the relationship between the TTC reduction and the O_2 consumption of the seedling roots was also highly significant.

Discussion

The TTC test is commonly used to distinguish living from dead fine roots, and to assess the vitality classes of the fine roots of woody and non-woody plants (Clemensson-Lindell 1994, Comas et al. 2000, Ruf and Brunner 2003, Sturite et al. 2004). In these investigations, boiled roots often served as controls. The reduction of TTC by the boiled roots was often found acceptably low and comparable to the reactivity of fine roots that were defined as dead (Ruf and Brunner 2003, Sturite et al. 2004). However, Clemensson-Lindell (1994) and Comas et al. (2000) observed that the reduction of TTC by boiled roots remained high. Our tests revealed that the boiled fine roots of adult trees reduced TTC as much as living fine roots. Dried and ground fine roots of adult trees showed the same reactivity with TTC.

By separating the bark from the wood of coarse roots, we were able to demonstrate that reactive substances occur in higher quantities in the bark than in the wood. It was shown that these substances could be largely eliminated by water extraction and, for smaller quantities, by a water:methanol extraction. Water and alcohol extractions are commonly used to extract plant polyphenolic substances (Peng et al. 1991, Matthews et al. 1997, Kraus et al. 2003). In wood-processing, boiling is a common method to extract polyphenols (Roffael et al. 2000). Pan and Lundgren (1995) detected polyphenols like epicatechin and catechin and other proanthocyanidins (condensed tannins) in the root bark of Norway spruce. Our data also revealed that the extractable components from spruce fine roots (15–29% dry mass; data not shown) were comparable in quantity to the extractable polyphenols measured by Matthews et al. (1997) (21% dry mass). Confirming that polyphenols are abundant in root bark, Kraus et al. (2003) reported that polyphenols reach up to 35% of the dry mass of roots.

To test if these polyphenolic components in the fine roots are able to reduce TTC, we compared the reactivity of the lyophilised water extract of spruce fine roots with several cell-wall constituents. The results demonstrated that root polyphenols, especially epicatechin and catechin, are as reactive with TTC as the fine root water extract. Due to their antioxidant and

Table 2. Mean triphenyltetrazolium chloride reactivity (absorbance of triphenyl formazan (TF) ($Abs_{520}\ g_{DM}^{-1}$) ± SE) of different root cell-wall constituents, polyphenols, dried and ground spruce fine roots, the water extract of the dried and ground spruce fine roots, and two controls (1 mg).

Substance class	Substance	Absorbance ($Abs_{520}g_{DM}^{-1}$)
Cell-wall constituents	Cellulose	35 ± 16
	Pectin	31 ± 14
	Lignin	310 ± 22
Polyphenols	Tannic acid	176 ± 111
	Ellagic acid	187 ± 14
	Catechin	293 ± 57
	Epicatechin	360 ± 65
Dried/ground fine roots	Powder	402 ± 71
	Lyophilised water extract	357 ± 126
Controls	Quartz sand	0.01 ± 0.13
	Water	0.2 ± 0.23

Table 3. Triphenyltetrazolium chloride reactivity (absorbance of triphenyl formazan (TF) ($Abs_{520}\ g_{DM}^{-1}$) of Norway spruce and European beech fine roots of different ages (age in months in parentheses) and origins, and the remaining percentage of the reactivity of the living roots. Different letters indicate a significant difference ($P < 0.05$).

Roots	Norway spruce				European beech		
	Forest		Greenhouse		Forest		
	Adult (> 6)	Seedlings (6)	Seedlings (2)	Seedlings (1)	Adult (> 6)	Seedlings (2)	Seedlings (1)
Living	196 a	460 a	364 a	672 a	280 a	446 a	348 a
Dead (boiled)	156 a	148 b	43 b	87 a[1]	220 a	89 b	37 b
Dead as % of living	80%	32%	12%	13%	79%	20%	11%

[1] $n = 2$.

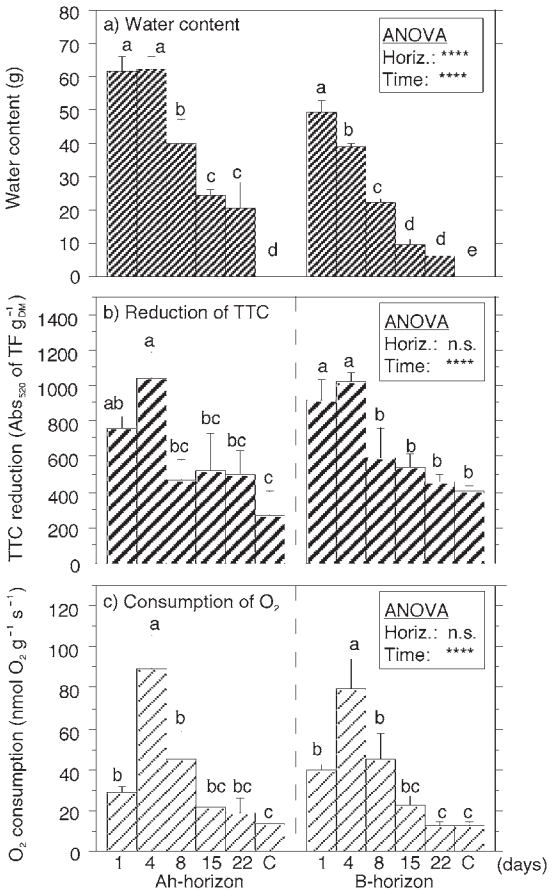

Figure 2. Changes over the course of a 22-day drought in the water content of the Ah and B horizons of soil monoliths with Norway spruce seedlings (a); and in the triphenyltetrazolium chloride (TTC) reactivity (b) and O_2 consumption (c) of the Norway spruce seedling roots. Different letters indicate a significant difference ($P < 0.05$) and bars indicate standard error. Abbreviations: C = control after 18 days of drought and 4 days drying at 50 °C; **** = $P < 0.0001$; and n.s. = not significant.

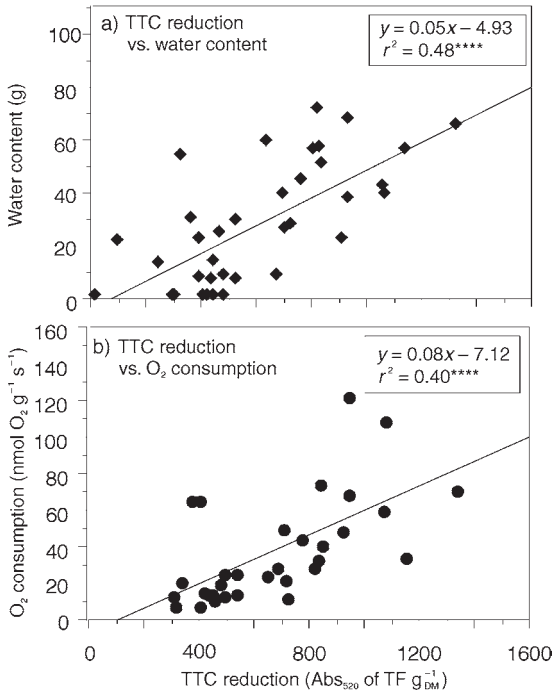

Figure 3. Relationship between triphenyltetrazolium chloride (TTC) reactivity of Norway spruce seedling roots after different lengths of drought: (a) with the water content of soil monoliths; and (b) with the consumption of O_2 of Norway spruce seedling roots. Probability level for the analysis of variance (ANOVA): **** = $P < 0.0001$.

radical scavenging ability, polyphenols are extremely reactive (Rice-Evans et al. 1997, Hättenschwiler and Vitousek 2000, Kraus et al. 2003, Karonen et al. 2004, Dixon et al. 2005) with related tetrazolium salts (Galato et al. 2001, Aehle et al. 2003, Franke et al. 2004). For example, Vyas et al. (2002) observed that XTT (sodium, 3´-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-*bis*(4-methoxy-6-nitro)benzene-sulfonic acid hydrate) is reduced by polyphenols, especially by catechin.

Nevertheless, reflecting their low polyphenol content, we found that boiling seedling roots resulted in a measurable reduction in their TTC reactivity. Beyeler and Heyser (1997) measured an increase in polyphenols in European beech seedlings with age. As a consequence, the TTC test can give a false indication of vitality in fine roots of mature trees.

To demonstrate the link between respiration (O_2 consumption) and TTC reduction, we monitored TTC reduction by Norway spruce seedlings roots during the imposition of drought while simultaneously measuring root oxygen consumption with a Clark-type O_2-electrode. Both measurements indicated a significant decrease in root vitality with increasing drought stress. The measurements were significantly correlated and thus appeared to be equally valid measures of root respiration. These results support the findings of Comas et al. (2000), that a good correlation exists between TTC reduction and O_2 consumption in the fine roots of young grape vines. That TTC reactivity of seedling roots, unlike O_2 consumption, did not approach zero toward the end of the 22-day drought can be explained by the presence of some polyphenolic substances even in seedling roots. Kirakosyan et al. (2004) reported a 6 to 10-fold increase in epicatechin and catechin in two hawthorn species (*Crataegus spp.*) in response to 10 days of drought.

In conclusion, our results indicate that the TTC test can be applied to estimate the vitality of fine roots of tree seedlings but not of adult tree fine roots, which have a high concentration of polyphenols. The boiled controls indicate that the TTC reactivity of living fine roots with a high polyphenolic content is not solely due to the respiratory activity of the tissue, but in part, depends on the presence of polyphenols. In this case, measurements of O_2 consumption with a Clark-type electrode may be a valuable alternative to indicate the vitality of fine roots.

Acknowledgments

This study was financed by the VELUX-Foundation. We thank Gregor Ernst for help with the soil monolith sampling, the group of Emmanuel Frossard for constructive discussions and Silvia Dingwall for

improving our English.

References

Aehle, E., S. Raynaud-Le Grandic, R. Ralainirina, S. Baltora-Rosset, F. Mesnard, C. Prouillet, J.-C. Mazière and M.-A. Fliniaux. 2003. Development and evaluation of an enriched natural antioxidant preparation from aqueous spinach (*Spinacia oleracea*) extracts by an absorption procedure. Food Chem. 86:579–585.

Bakker, M.R. 1999. Fine-root parameters as indicators of sustainability of forest ecosystems. For. Ecol. Manage. 122:7–16.

Beyeler, M. and W. Heyser. 1997. The influence of mycorrhizal colonization on growth in the greenhouse and catechin, epicatechin and procyanidin in roots of *Fagus sylvatica* L. Mycorrhiza 7:171–177.

Blaser, P., M. Zysset, S. Zimmermann and J. Luster. 1999. Soil acidification in southern Switzerland between 1987 and 1997: a case study on the critical load concept. Environ. Sci. Technol. 33: 2382–2389.

Brunner, I., M. Ruf, P. Lüscher and C. Sperisen. 2004. Molecular markers reveal extensive intraspecific below-ground overlap of silver fir fine roots. Mol. Ecol. 13:3595–3600.

Clemensson-Lindell, A. 1994. Triphenyltetrazolium chloride as an indicator of fine-root vitality and environmental stress in coniferous forest stands: applications and limitations. Plant Soil 159: 297–300.

Clemensson-Lindell, A. and H. Persson. 1995. Fine-root vitality in a Norway spruce stand subjected to various nutrient supplies. Plant Soil 168/169:167–172.

Comas, L.H. and D.M. Eissenstat. 2004. Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. Funct. Ecol. 18:388–397.

Comas, L., D.M. Eissenstat and A.N. Lakso. 2000. Assessing root death and root system dynamics in a study of grape canopy pruning. New Phytol. 147:171–178.

Dixon, R.A., D.-Y. Xie and S.B. Sharma. 2005. Proanthocyanidins—a final frontier in flavonoid research? New Phytol. 165:9–28.

Franke, S.I.R., K. Ckless, J.D. Silveira, G. Rubensam, M. Brendel, B. Erdtmann and J.A.P. Henriques. 2004. Study of antioxidant and mutagenic activity of different orange juices. Food Chem. 88: 45–55.

Galato, D., K. Ckless, M.F. Susin, C. Giacomelli, R.M. Ribeiro-do-Valle and A. Spinelli. 2001. Antioxidant capacity of phenolic and related compounds: correlation among electrochemical, visible spectroscopy methods and structure-antioxidant activity. Redox Report 6:243–250.

Godbold, D.L., H.W. Fritz, G. Jentschke, H. Meesenburg and P. Rademacher. 2003. Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. Tree Physiol. 23:915–921.

Graf Pannatier, E., J. Luster, S. Zimmermann and P. Blaser. 2005. Acidification of soil solution in a chestnut forest stand in southern Switzerland: are there signs of recovery? Environ. Sci. Technol. 39: 7761–7767.

Hättenschwiler, S. and P.M. Vitousek. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. Trends Ecol. Evol. 15: 238–243.

Helmisaari, H.S. and L. Hallbäcken. 1999. Fine-root biomass and necromass in limed and fertilized Norway spruce (*Picea abies* (L.) Karst) stands. For. Ecol. Manage. 119:99–110.

Hertel, D. and C. Leuschner. 2002. A comparison of four different fine root production estimates with ecosystem carbon balance data in a *Fagus–Quercus* mixed forest. Plant Soil 239:237–251.

Hodge A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 162:9–24.

Jentschke, G., M. Drexhage, H.-W. Fritz et al. 2001. Does soil acidity reduce subsoil rooting in Norway spruce (*Picea abies*)? Plant Soil 237:91–108.

Karonen, M., M. Hämäläinen, R. Nieminen, K.D. Klika, J. Loponen, V.V. Ovcharenko, E. Moilanen and K. Pihlaja. 2004. Phenolic extractives from the bark of *Pinus sylvestris* L. and their effects on inflammatory mediators nitric oxide and prostaglandin E_2. J. Agric. Food Chem. 52:7532–7540.

Kirakosyan, A., P. Kaufmann, S. Warber, S. Zick, K. Aaronson, S. Bolling and C. Chang. 2004. Applied environmental stress to enhance the levels of polyphenolics in leaves of hawthorn plants. Physiol. Plant. 121:182–186.

Kraus, T.E.C., R.A. Dahlgren and R.J. Zasoski. 2003. Tannins in nutrient dynamics of forest ecosystems—a review. Plant Soil 256: 41–66.

Matthews, S., I. Mila, A. Scalbert and D.M.X. Donnelly. 1997. Extractable and non-extractable proanthocyanidins in barks. Phytochemistry 45:405–410.

Pan, H. and L.N. Lundgren. 1995. Phenolic extractives from root bark of *Picea abies*. Phytochemistry 39:1423–1428.

Peng, S., A. Scalbert and B. Monties. 1991. Insoluble ellagitannins in *Castanea sativa* and *Quercus petraea* woods. Phytochemistry 30: 775–778.

Rice-Evans, C.A., N.J. Miller and G. Paganga. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2:1360–1385.

Roffael, E., B. Dix and J. Okum. 2000. Use of spruce tannin as a binder in particleboards and medium density fibreboards (MDF). Holz als Roh- und Werkstoff 58:301–305.

Ruf, M. and I. Brunner. 2003. Vitality of tree fine roots: reevaluation of the tetrazolium test. Tree Physiol. 23:257–263.

Stattin, E. and A. Lindström. 1999. Influence of soil temperature on root freezing tolerance of Scots pine (*Pinus sylvestris* L.) seedlings. Plant Soil 217:173–181.

Sturite, I., T.M. Henriksen and T.A. Breland. 2004. Distinguishing between metabolically active and inactive roots by combined staining with 2,3,5-triphenyltetrazolium chloride and image colour analysis. Plant Soil 271:75–82.

Vanguelova, E.I., S. Nortcliff, A.J. Moffat and F. Kennedy. 2005. Morphology, biomass and nutrient status of fine roots of Scots pine (*Pinus sylvestris*) as influenced by seasonal fluctuations in soil moisture and soil solution chemistry. Plant Soil 270:233–247.

Vyas, D., R. Sahoo and S. Kumar. 2002. Possible mechanism and implications of phenolics-mediated reduction of XTT (sodium, 3′-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-*bis*(4-methoxy-6-nitro)benzene-sulfonic acid hydrate). Current Sci. 83:1588–1592.

Zhu, X.B., R.M. Cox, C.-P.A. Bourque and P.A. Arp. 2002. Thaw effects on cold-hardiness parameters in yellow birch. Can. J. Bot. 80: 390–398.