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The influence of cellulose content on tensile strength in tree roots

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Abstract

Root tensile strength is an important factor to consider when choosing suitable species for reinforcing soil on unstable slopes. Tensile strength has been found to increase with decreasing root diameter, however, it is not known how this phenomenon occurs. We carried out tensile tests on roots 0.2–12.0 mm in diameter of three conifer and two broadleaf species, in order to determine the relationship between tensile strength and diameter. Two species, *Pinus pinaster* Ait. and *Castanea sativa* Mill., were then chosen for a quantitative analysis of root cellulose content. Cellulose is responsible for tensile strength in wood due to its microfibrillar structure. Results showed that in all species, a significant power relationship existed between tensile strength and root diameter, with a sharp increase of tensile strength in roots with a diameter <0.9 mm. In roots >1.0 mm, *Fagus sylvatica* L. was the most resistant to failure, followed by *Picea abies* L. and *C. sativa*., *P. pinaster* and *Pinus nigra* Arnold roots were the least resistant in tension for the same diameter class. Extremely high values of strength (132–201 MPa) were found in *P. abies*, *C. sativa* and *P. pinaster*, for the smallest roots (0.4 mm in diameter). The power relationship between tensile strength and root diameter cannot only be explained by a scaling effect typical of that found in fracture mechanics. Therefore, this relationship could be due to changes in cellulose content as the percentage of cellulose was also observed to increase with decreasing root diameter and increasing tensile strength in both *P. pinaster* and *C. sativa*.

Introduction

The use of vegetation by civil engineers when dealing with unstable slopes has become increasingly popular over the last 20 years (Bischetti et al., 2006; Coppin and Richards, 1990; Gray and Sotir, 1996; Greenway, 1987; Norris, 2005; Roering et al., 2003; Schiechtl, 1980). In particular, trees and woody shrubs have been studied with regards to the soil reinforcing properties that their root systems convey to slopes subject

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to erosion or slippage problems (Schmidt et al., 2001; Wu, 2006). If the root system characteristics, which govern soil stabilization, could be better identified, screening of suitable species for use on unstable slopes would be more efficient.

Vegetation has been recognized as a factor useful for increasing the shear resistance of soil on an unstable slope (Anderson and Richards, 1987; Coppin and Richards, 1990; Operstein and Frydman, 2000). The major factors which influence the shear resistance of root-permeated soil are the quantity and directional distribution of roots as well as their tensile strength, soil shear strength and soil–root interaction. Strength is the maximum force per unit area required to cause a material to break (Niklas, 1992). Tensile strength is considered one of the most important factors governing soil stabilization and fixation, and has therefore been studied in great detail (Burroughs and Thomas, 1977; Hathaway and Penny, 1975; Nilaweera and Nutalaya, 1999; Operstein and Frydman, 2000; Phillips and Watson, 1994; Schiechtl, 1980). Not only is root tensile strength important when considering soil reinforcement, but can also affect plant anchorage. In herbaceous species, plants must withstand grazing pressure, whereby uprooting occurs in tension, therefore a higher root tensile strength will enable the plant to remain anchored in the soil (Ennos and Fitter, 1992). In trees, most anchorage is provided by the large structural roots (Stokes, 2002), however, the roots held in tension provide around 60% of the resistance to overturning during a storm (Coutts, 1983). Therefore, a greater root tensile strength will also be beneficial for tree anchorage.

Wide variations in root tensile strength have been reported in the literature, and appear to depend on species and site factors such as the local environment, season, root diameter, and orientation (Gray and Sotir, 1996). Root resistance to failure in tension can be influenced by the mode of planting e.g. naturally regenerated Scots pine (*Pinus sylvestris* L.) had stronger roots than those of planted pines (Lindström and Rune, 1999). The time of year has also been found to affect tensile strength, roots being stronger in winter than in summer, due to the decrease in water content (Turmanina, 1965). Tensile strength usually decreases with increasing root size (Burroughs and Thomas, 1977; O'Loughlin and Watson, 1979; Operstein and Frydman, 2000; Turmanina, 1965; Wu, 1976) and this phenomenon has been attributed to differences in root structure, with smaller roots possessing more cellulose per dry mass than larger roots (Commandeur and Pyles, 1991; Hathaway and Penny, 1975; Turmanina, 1965).

The structure of cellulose has been found to be optimal for resisting failure in tension (Sjostrom, 1993). Cellulose is made up of polymer chains consisting of glucose units which are linked together by highly resistant hydrogen bonds (Delmer and Amor, 1995). These cellulose chains are then grouped together in a hemicellulose matrix and the entire structure is termed a microfibril. Each layer of the wood cell wall is made up of many microfibrils arranged in a helical structure.

In order to determine the relationship between tensile strength for a range of species and root size, mechanical tests were carried out on small roots from three conifer and two broadleaf species. To relate the root strength to the cellulose content, two species were then chosen for subsequent dosing of percentage cellulose in those roots tested mechanically. Results are discussed with regards to the structure of cellulose.

Materials and methods

Plant material

Roots with a diameter between 0.2 and 12.0 mm were collected from five tree species (Table 1). Trees were situated throughout different parts of France (Table 1). Roots of Maritime pine, Austrian pine and Sweet chestnut were collected from a sandy podzol soil in Gironde, located in SW France (Cucchi et al., 2004). Trees were growing at an altitude of 58 m in a flat region, where mean annual precipitation is 990 mm. Norway spruce and Sweet chestnut roots were sampled in the Forêt domaniale de Vaujany, Isère, in the French Alps. This forest, which is located at an altitude of 1350–1600 m, has a slope gradient of 38–42°. The soil is a crystalline soil and mean annual precipitation is 1353 mm (Stokes et al., 2005). Species were chosen in such a way as to cover a broad range of roots to test from both conifer

Table 1. Location of the different species used in the tensile tests and parameters of the trees and the roots tested

Species common name and Latin name	Location where collected in France	Number of trees	Min.–Max. Height (m)	Min.–Max. DBH (m)	Total number of roots sampled	Number of roots successfully tested
Austrian pine (*Pinus nigra* Arnold)	Gironde	2	15.3–17.6	0.3–0.49	85	30
Maritime pine (*Pinus pinaster* Ait.)	Gironde	2	33.0–36.2	0.28–0.4	81	34
Norway spruce (*Picea abies* L.)	Isère	3	10.7–14.6	0.19–0.26	91	27
European Beech (*Fagus sylvatica* L.)	Isère	2	15.7–17.8	0.18–0.27	35	11
Sweet chestnut (*Castanea sativa* Mill.)	Gironde	2	NA	NA	202	53

NA–not available as trees were coppiced.

and broadleaf trees. Roots were collected from two or three trees for each species (Table 1).

Live roots were manually excavated to a depth of about 0.6–0.7 m below the soil surface. Care was taken to avoid any damage to roots during the excavation process. Samples were collected randomly from the root system in order to have representative samples of different types of roots. Once the roots had been removed from the tree, they were put into separate bags and taken to the laboratory where they were stored at 4 °C. Mechanical testing was carried out as soon as possible, always within 1 week from sampling, to ensure that root material was still fresh.

Root tensile tests

Tensile testing was carried out on 494 root samples, using a Universal Testing machine (ADAMEL Lhomargy, France). The length of each sample was at least 15 times its central diameter. A load cell with a maximal capacity of 1.0 kN was used to measure the force required to cause failure in tension of each root. Crosshead speed was kept constant at 2.0 mm min^{-1} and both force and speed were measured constantly via a PC during each test. In order to avoid slippage of roots out of the clamps (Nilaweera and Nutalaya, 1999), thin slices of cork were inserted between the jaws and the root. The cork helped to improve the grip between the jaws and the root. Tests were considered successful only when specimens failed approximately in the middle of the root so that root rupture was due to the force applied in tension and not due to any existing damage (Table 1).

Tensile strength was calculated as the maximal force required to cause failure in the root, divided by the root cross-sectional area (CSA) at the point of breakage. The diameter of each root was measured with an electronic slide gauge with 1/50 mm accuracy.

Cellulose content

Two contrasting species were chosen for consequent measurements of cellulose content: Maritime pine and Sweet chestnut. The method used to measure total cellulose content was based on that developed by Leavitt and Danzer (1993) and consisted of removing as many non-cellulosic compounds as possible from the root material. Initially, bark was removed from each root using a scalpel. The roots were then dried at 60 °C for 24 h and weighed using a balance with a precision >0.001 mg. Each root was then ground into a fine powder with a vibration mill (Retsch MM 300). This powder was poured into a Teflon sachet (no. 11803, pore size 1.2 μm), and each bag was carefully marked with the identification code of the corresponding root. Teflon sachets were used because they have a good compatibility with strong acids and solvents and are resistant to heat with inflammable temperatures around 200 °C (Lambrot and Porté, 2000).

The first compounds removed from the ground root tissue were lipids (waxes, oils and resins). Each sample was placed into a soxhlet extractor (50-mm *i.d.*, 200 mL capacity to siphon top) equipped with a flask containing a 700 mL mixture of toluene 99%–ethanol 96% (2–1; v/v) heated until boiling point. After 24 h of extraction using this method, the toluene ethanol was replaced with 700 mL of ethanol heated to the same temperature. After 24 h, the samples were removed from the soxhlet and immersed in distilled water heated to 100 °C for 6 h. This process removes hydrosoluble molecules from the sample.

The final step consisted of eliminating lignin compounds from the samples. Each sample was placed in a beaker containing 700 mL of distilled water, 7.0 g of sodium chlorite ($NaClO_2$), and 1.0 mL of acetic acid ($C_2H_4O_2$). The samples and solution was shaken using a magnetic agitater and heated to 60–70 °C during 12 h. This procedure was repeated three times, with the solution concentrated by 100% each time. The samples were then removed and rinsed in distilled water, dried at ambient temperature during 12 h and weighed. The percentage of cellulose was evaluated by calculating the relative difference in the initial and final weight of each sample.

Statistical analyses

Linear and power regressions were carried out initially to evaluate the correlation between the different variables. A Kolmogorov–Smirnov test was used to test the normality of the data before proceeding with analyses of variance. Data were log-transformed, before analysis, to reflect the power relationship in linear regressions. To evaluate the influence of species, diameter of roots and cellulose content on tensile strength of roots, analysis of covariance (ANCOVA) and analysis of variance (ANOVA) were used. ANCOVA was used to detect differences in cellulose content of roots between species with regards to root diameter. In order to evaluate the influence of species on tensile strength only, roots were then classed into two groups according to diameter (<0.9 mm and >1.0 mm) and a Student's *t*-test was carried out to detect differences in tensile strength between the two groups. These data were then analyzed with ANOVA and pair wise Tukey's Studentized Range (HSD) test in order to determine differences between species. Data were analyzed with Minitab version 13 or XLstat-Pro version 7.5 software.

Results

Root tensile tests

Only 33% of the tensile tests were successful (Table 1). Failure often occurred near the jaws, or roots slipped out of the clamps. Mean root tensile strength was significantly different between species ($F_{4,\ 152} = 15.16$, $p < 0.001$, ANCOVA) with regards to root diameter ($F_{1,\ 155} = 113.01$, $p < 0.001$, ANCOVA). Mean root strength was 28.4 ± 2.0 MPa when all species and diameters were considered together (means are ± standard error). A power regression between tensile strength and diameter was significant for all species (Table 2, Figure 1). Tensile strength was also significantly different between root size classes ($t = 5.49$, $p < 0.001$). For roots <0.9 mm, mean tensile strength for each species was greater than for roots >1.0 mm but variability was high (Figure 1). However, when root size classes were analyzed individually, no significant differences were found between species for roots <0.9 mm (ANOVA). Nevertheless, extremely high values of strength (132–201 MPa) were found in Norway spruce, Maritime pine and Sweet chestnut, for this size class of roots (Figure 1). For roots >1.0 mm, the tensile strength of roots was significantly different between species ($F = 10.17$, $p < 0.001$, ANOVA/HSD). Within this root size class, European beech was found to be the most resistant to failure in tension, followed by Norway spruce and Sweet chestnut. Maritime pine and Austrian pine roots were the least resistant in tension for the same diameter class.

Table 2. Parameters of the root tensile strength and diameter power law regressions for each tree species tested

Species	Regression Equation	R^2	p
Austrian pine	$y = 18.40x^{-0.52}$	0.23	0.010
Maritime pine	$y = 23.40x^{-0.87}$	0.51	<0.001
Norway spruce	$y = 37.86x^{-0.51}$	0.43	0.005
European Beech	$y = 63.51x^{-0.61}$	0.56	0.006
Sweet chestnut	$y = 31.92x^{-0.73}$	0.51	<0.001

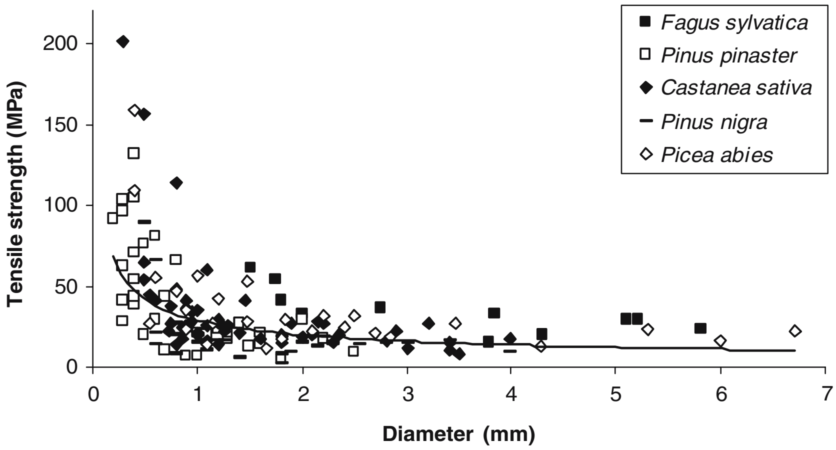

Figure 1. Tensile strength increased significantly with decreasing diameter when roots of Sweet chestnut, European beech, Maritime pine, Austrian pine and Norway spruce were considered together ($y = 28.97x^{-0.52}$, $R^2 = 0.30$, $p < 0.001$).

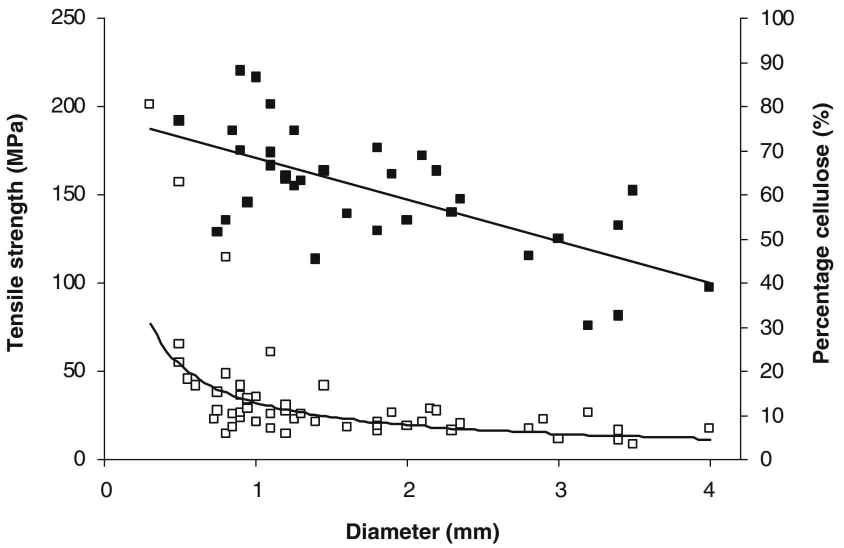

Figure 2. Tensile strength (white squares, Table 2) and cellulose content (black squares, $y = -9.44x + 77.59$, $R^2 = 0.43$, $p < 0.001$) decreased significantly with increasing root diameter in roots of Sweet chestnut.

Cellulose content

Maritime pine and Sweet chestnut roots were chosen for subsequent dosing of cellulose content, as a higher number of samples were available across the entire diameter range. The mean cellulose content was 60.0 ± 2.2% in Sweet chestnut roots and 69.9 ± 2.3% in Maritime pine roots. Cellulose content of roots was significantly different according to diameter ($F_{1,68} = 49.8$, $p < 0.001$, ANCOVA) but was not different between the two species ($F_{1,\ 68} = 0.32$, $p = 0.58$, ANCOVA). As with tensile strength, a significant linear relationship existed between cellulose content and root diameter for both Sweet chestnut (Figure 2) and Maritime pine ($y = -13.49 + 81.87$, $R^2 = 0.34$, $p < 0.001$). Root tensile strength was also significantly related to

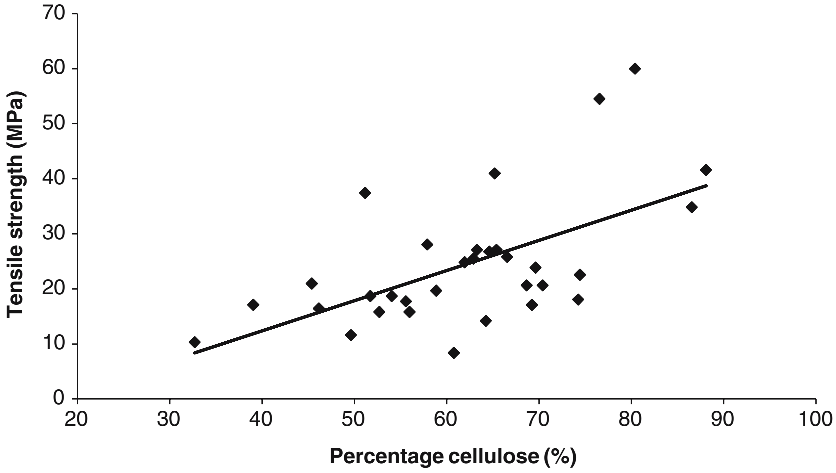

Figure 3. Tensile strength was significantly and positively related to percentage cellulose in roots of Sweet chestnut ($y=0.56x$-9.45, $R^2 = 0.34$, $p<0.001$).

cellulose content, however, variability was high in both Sweet chestnut (Figure 3) and Maritime pine ($y=0.95x-24.48$, $R^2=0.17$, $p=0.026$).

Discussion

Results from the tensile testing of roots were comparable to those of other authors on woody species, in that a power equation existed between diameter and tensile strength (Burroughs and Thomas, 1977; Gray and Sotir, 1996; Nilaweera and Nutalaya, 1999; O'Loughlin and Watson, 1979; Operstein and Frydman, 2000; Turmanina, 1965; Wu, 1976). The smallest roots were the most resistant in tension, and strength increased sharply with a decrease in root diameter <0.9 mm. Tensile strength differed between the species tested, for roots >1.0 mm, with beech being the most resistant, followed by Norway spruce, Sweet chestnut, Maritime and Austrian pine. Values for roots >1.0 mm are similar to those reported in previous studies for Maritime pine and Norway spruce (Bischetti et al., 2006; Turmanina, 1965). For roots <0.9 mm, no significant differences in tensile strength between species were observed, probably due to the low number of samples available. A comparison with other studies is not possible since, to our knowledge, no other studies exist concerning the tensile strength of such small roots for any of the species tested. The strength values of 132–201 MPa observed in Norway spruce, Maritime pine and Sweet chestnut were surprising, as such high values have rarely been documented in the literature. These results may be due to the fact that such small tree roots are rarely tested. To our knowledge, only Operstein and Frydman (2000) and Bischetti et al. (2006) have carried out tensile tests on small diameter roots. In the species tested by Operstein and Friedman (2000), only woody shrubs were measured and values were always lower than 80 MPa. However, Bischetti et al. (2006) also found extremely high values in roots 0.2–0.5 mm in diameter. These authors observed tensile strength values up to 750 MPa in several tree species, including beech and Norway spruce located in the Prealps. Therefore, strength values tend to lie within the range typical of that usually reported for tree roots (Schiechtl, 1980; Stokes, 2002; Ziemer, 1981) with the only exceptions being for very small diameter roots. It would be of extreme interest to carry out more testing of such small diameter roots, and to determine why tensile strength values may be so high in certain roots.

Not only is root tensile strength an important parameter to consider when determining the influence of vegetation on slope reinforcement (Greenwood et al., 2001), but is also an important factor with regards to tree anchorage (Coutts, 1983). It would therefore be interesting to relate root tensile strength to tree resistance to overturning. Winching tests were carried out on Norway spruce and European beech by Stokes et al. (2005) on the same trees where root samples were collected for our study. Trees were winched sideways and the force necessary to cause failure was measured. The critical turning moment TM_{crit} was then calculated (Cucchi et al., 2004). Results showed that European beech was significantly more resistant to overturning than Norway spruce. As the tensile strength of beech roots > 1.0 mm was higher than that of Norway spruce roots, it may be assumed that this mechanical property plays an important role in tree resistance to overturning. It would be of extreme interest to study in detail the correlation between TM_{crit} and root tensile strength in order to evaluate the importance of this parameter on tree anchorage.

A power relationship, $\sigma_n \approx d^{-a}$, with $\alpha \geq 0.5$, existed between root tensile strength σ_n and diameter d. This type of relation is well-known in fracture mechanics as a size effect between small and large samples (Bazant and Kazemi, 1990). The size effect is transitional between two asymptotic behaviors. There is no size effect for small dimensions of structures. For bigger dimensions a power relationship exists between the nominal strength σ_n and a characteristic dimension of the structure, e.g. the root diameter d, $\sigma_n \approx d^{-a}$ which is the size effect exhibited by Linear Elastic Fracture Mechanics (Bazant and Kazemi, 1990). Therefore the exponent term α cannot be greater than 0.5. However, our results show that this exponent exceeded systematically this maximum theoretical value. This was also the case in previous studies on root tensile strength (Bischetti et al., 2006; Gray and Sotir, 1996; Operstein and Frydman, 2000). These differences between theoretical and experimental equations could be due to experimental error, but the estimated exponent value always overestimated the maximum theoretical exponent value. Another possible explanation for our results is that the wood material is different according to root size. This assumption was confirmed by the observed change in cellulose content between the samples.

The quantity of cellulose was found to differ significantly between roots of different sizes as well as between Sweet chestnut and Maritime pine. When both species were considered together, the mean cellulose content of roots was 65%. The mean percentage cellulose in roots was therefore in the same range as other values found in the literature e.g. Hathaway and Penny (1975) found that mean cellulose percentage in roots of six *Populus* and *Salix* species was 72%. Cellulose quantity and tensile strength of roots were significantly correlated but variability was high. In our study, cellulose content was measured using the method developed by Leavitt and Danzer (1993). In this method, hemicelluloses, which are polysaccharides linked to the cellulose present in the cell walls, were not separated from the crystalline cellulose. The quantity obtained at the end of the experiment represents therefore both cellulose and hemicelluloses. The amount of hemicelluloses of the dry weight of wood is usually around 20%. Hathaway and Penny (1975) separated hemicelluloses and crystalline cellulose. These authors found that hemicelluloses represent 17% of the dry weight of wood in roots studied. However, the hemicellulose content and composition differs between species (Sjostrom, 1993). The changes in these proportions may therefore be able to explain the high variability observed in our results. A further experiment whereby only crystalline cellulose was measured would help determine the influence of cellulose content on wood tensile strength (Akerholm et al., 2004; Andersson et al., 2003). Other chemical and anatomical parameters, which can influence tensile strength of roots, should explain the high variability observed. Lignin can also affect strength properties, especially at high moisture contents (Hathaway and Penny, 1975). The microfibril angle in root wood may also influence mechanical properties (Kerstens et al., 2001). When these microfibrils are aligned at an angle almost parallel to the cell axis, as in young wood, the combined effect of these cellulose chains is a high resistance in tension, but a low bending strength (Archer, 1986; Sjostrom, 1993). Thus, future work should concentrate on the influence of microfibril angle and lignin/cellulose ratio on tensile strength of roots.

Although cellulose content and tensile strength increases with decreasing root diameter, no measurements of annual growth rings were made in the roots studied, therefore the age of each root remains unknown. It can be imagined that cellulose content is higher in young roots, which are more resistant in tension, but this assumption should be verified through measurements of root age.

Differences in cellulose content have been proposed as the major determinant governing root tensile strength (Commandeur and Pyles, 1991; Turmanina, 1965). Nevertheless, the shape and size of a root system is influenced by its immediate environment as well being inherent to a particular species (Köstler et al., 1968). For example, trees growing on slopes may develop a specific type of root system architecture, as the mechanical function of the uphill portion of the root system is different to that downhill (Chiatante et al., 2003; Köstler et al., 1968; Shrestha et al., 2000). Root system morphology can also be modified by soil type. Nutrient supply, fertility and soil acidity all influence root growth (Fitter and Stickland, 1991; Gersani and Sachs, 1992; Gruber, 1994). Soil physical properties such as soil bulk density and strength are also important factors affecting both shoot and root growth (Campbell and Hawkins, 2003; Goodman and Ennos, 1999). In our study, samples were collected from two different habitats. As root morphology is affected by local environment and since root chemical composition also varies with root morphology, it may be possible that the local environment also influenced root cellulose content. More studies on the differences in root tensile strength of species from the same site are therefore necessary. It would also be of interest to compare the tensile strength of roots from trees growing on different types of slope or in different soil conditions, as well as testing cellulose content and tensile strength in roots around a tree, and to compare up- and downhill roots growing on a slope (Schiechtl, 1980). Not only can cellulose content be assumed to differ between roots in a root system, but the role of this chemical compound in the overall anchorage of a root system needs to be determined, especially in young trees or woody shrubs. It has generally been assumed that root architecture is the principal component in resisting uprooting of a plant (Ennos, 2000; Dupuy et al., 2005, 2006; Hamza et al., 2006; Stokes et al., 2000). However, a highly branched root system will probably not have the same percentage cellulose as a root system with fewer but thicker branches. The role each parameter plays in resisting uprooting therefore needs to be investigated.

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