The Influence of Steep Slopes on Root System Development

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ABSTRACT

Mechanical forces active on steep slopes tend to overturn plants, which respond by developing a specific asymmetrical architecture in the root system. This asymmetric architecture is the consequence of preferential lateral root emergence and elongation in the up-slope and down-slope directions. Root systems show a normal symmetrical architecture when the same species is grown on plane soil. The asymmetrical root architecture on steep slopes seems to increase the plant's stability by modifying the distribution of mechanical forces into the soil. This hypothesis is supported by the observation that lateral roots developing in the up-slope or down-slope directions present considerable anatomical modifications in shape and tissue-organization compared with lateral roots from plants growing on plane soil.

INTRODUCTION

Molise is a small region situated in the center of Italy and characterized by a hilly countryside subjected to thousands of slide-prone slopes. The aim of our work was to explore the concept of root system adaptation to slope conditions to rank native plant species according to specific below-ground rooting habits that can be used to achieve good anchorage even when grown on a slope. We hope that an improvement of the native vegetation stability on steep slopes will have the secondary effect of in-creasing soil stability in slide-prone areas (O'Loughlin and Ziemer 1982; Phillips and others 1990; Sidle 1991; Wu and Erb 1988).

The mechanics of plant anchorage have not received great attention, and this is probably due to the fact that it seems intuitively obvious that friction or adhesion between the roots and the soil resists the uprooting of a plant (Ennos 1994). Furthermore, a second limitation of this research is the fact that the few studies on plant anchorage have focused mostly on the structure and function of the taproot whereas the development of lateral roots has received scarce attention. For example, studies on root orientation have concentrated more on taproots and seminal root response than on that of lateral ones (Coutts and Nicoll 1991). We believe that, as valuable as it is, the information accumulated in the literature on taproots is insufficient to

understand the complexity of plant anchorage, which ultimately depends on the collaborative function of all the roots present in the root system. Moreover, the function of the taproot in plant anchorage decreases with time: at an earlier stage of plant development the taproot is the most vigorous root within the root system but its importance decreases as that of the lateral roots increase (Coutts and Nicoll 1991). This decrease of taproot importance in plant anchorage is common to both broadleaved species (Lyford 1980) and conifers (Preisig and others 1979).

These considerations clearly indicate that research on plant anchorage should investigate the entire root system. Another topic to be considered in studies on plant anchorage is root system architecture, definable as the result in space of simultaneous processes of axial and radial growth and branching (Thaler and Pagès 1996). Obviously the important problem that the root system grows within an opaque, heavy, and coherent medium must be somehow solved (Pagès 2001). At present, there are no alternative methods to hand excavation for recovering intact root systems. The use of rhizotrons in these studies is limiting because it does not allow us to follow the growth of all the roots contemporaneously and in all the directions in space. In our studies we use hand excavation and we investigate the intact root system of plants growing on slopes with the aim of (1) understanding the distribution of mechanical forces in the root system; (2) identification of a specific root system architecture phenotype induced by slope, independent of species; (3) understanding the influence of late lateral root emergence on anchorage; (4) measuring the quantitative and/or qualitative variation in root traits such as diameter, shape, branching, and stiffness induced by the mechanical stimulus; (5) investigating a possible signal transduction pathway, which might be at the basis of the response of the root system to the slope. The following paper outlines our results to date and discusses them in the context of the literature on anchorage biomechanics.

ASYMMETRIC DISTRIBUTION OF MECHANICAL FORCES

To achieve anchorage, a plant transfers the loading forces experienced by the stem into the ground via roots. In the absence of any other environmental or artificial factor, the weight of the aerial part of the plant induces a mechanical stress on the root system and tends to overturn the plant. The effect of weight has been named ''*static loading*'' (Stokes and Guitard 1997), but in that case the authors referred to a situation where the weight forces were distributed on a plane soil instead of a slope soil, as in our case. The slope is a very complex environmental condition, not yet characterized, that subjects plants to several mechanical stresses. The weight of the stem and soil, and the strains of moving soil are some of the mechanical stresses associated with slope that strongly affect plant stability. No terms are reported in the literature for the weight forces acting on roots growing on slope conditions. For this reason, the mechanical stimulus due to the weight of a plant growing on a slope will be called here ''*self-loading*''. In this paper we will give particular attention to the ''self-loading'' forces, leaving apart other stresses of slope and other mechanical stimuli that could be superimposed on it and that might greatly influence root system development such as wind, touch, and mechanical impedance.

The mechanical aspect of plant design has not received much attention during the past one hundred years (Ennos 1994), but in the case of the root system it is not unreasonable to consider root system architecture as the network of directions followed by ''self-loading'' forces during their transfer to the ground. From a biomechanical point of view, it is not difficult to understand that a large root surface area dissipates ''self-loading'' more rapidly than a smaller one. A large surface area can be achieved by either larger or more highly branched roots. The former will ensure a greater resistance to bending forces (bending resistance is proportional to the fourth power of radius), whereas the latter will allow a rapid transferral of tensile forces into the soil (Stokes and Guitard 1997).

The indirect measurement of the distribution of forces on the root system of plants growing on a slope can be obtained from the analysis of gross root architecture (Nicoll and Ray 1996). With this purpose, we measured in 20–30-year-old *Quercus pubescens* the location of the center of the first-order lateral root cross-sectional area (CSA) at different distances with respect to the center of the stump (the taproot cross-sectioned to its stem/root collar). The center of the CSA is equivalent to the center of the mass described by Coutts and others (1990). In plants growing on a slope, the center of the CSA of the first-order laterals (the most important for anchorage) is asymmetrically located with respect to the center of the stump. In particular, the highest values for the CSA are located asymmetrically within a distance of 20–40 cm from the center of the trunk stump in the up-slope direction (Figure 1).
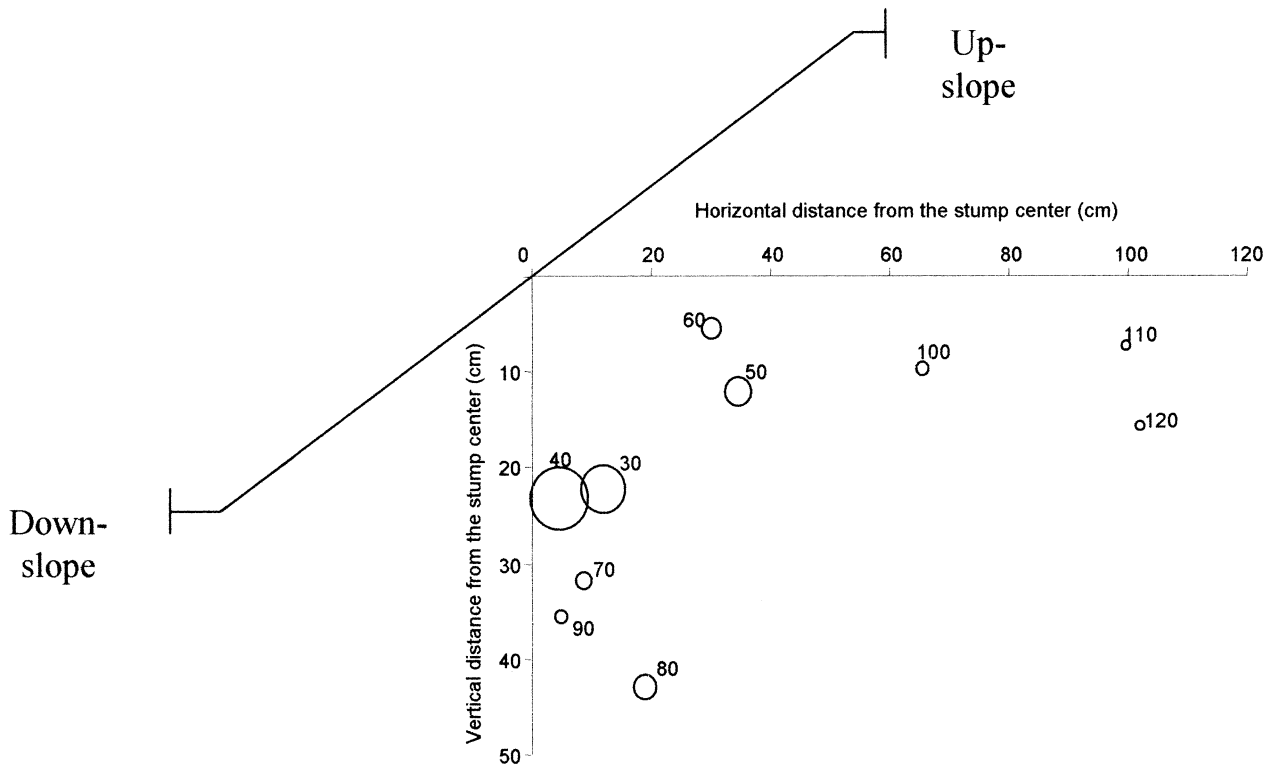
Figure 1. Two-dimensional representation of the centers of first-order lateral root cross-sectional areas (CSA). The CSA was measured every 10 cm at radial distances from the tree stump center. The horizontal axes is parallel to the maximum slope direction, drawn with a line passing through the center. Symbol size is proportional to the CSA. The coordinates of each point are the vector sum of the Cartesian coordinates (x_i, y_i, z_i) of the ith root ($i = 1...n$) weighted by its CSA.

Therefore this asymmetrical location of the CSA is another sign that the mechanical function of the up-slope portion of the root system is different from the down-slope function. This may be in response to a different "self-loading" force distribution.

In spite of the knowledge of biomechanics principles, and of the importance of vegetation in slope stability (Gray and Sotir 1996), the mechanical characteristics of the root system in plants growing on a slope have never been thoroughly investigated (Gilroy and others 2001). Even simple measurements of tensile strength have never been conducted on the root system of a plant growing on a slope (Ennos 1994). We predicted that the differences in the "self-loading" force distribution of lateral roots induces differences in the mechanical properties of the wood in such laterals. These different properties can be detected by examining root responses when an artificial force is applied to the stem. In our work we used a strain indicator (unpublished) to measure the strain response of some of the most superficial first-order laterals of two 20–30-year-old native tree species, *Quercus cerris* L. and *Quercus pubescens* Willd. With this purpose, we applied a force (tree pulling) to the stem in various

directions, and tested the strain response in different lateral roots. In the case of a plant growing on a slope, the force is applied to the stem (at breast height) along four directions: up-slope, down-slope, right, and left along a line that is perpendicular to the up-slope/down-slope direction. The measurements were repeated on 10 plants for each species. The results so far obtained clearly indicate that when plants grow on a slope, the lateral roots developing in the down-slope direction respond with a different degree of deformation than the laterals developing in the up-slope direction. In the case of plants growing on a plane, all the first order laterals respond to the strain test with similar deformations independently from the direction where the force is applied. Moreover, the deformations measured in roots of plants growing on a slope are always higher than that of plants growing on a plane. The response to the strain indicator tests suggests that in the examined root system, the wood of lateral roots has different biomechanical properties that seem to be related, at least, to two factors: (1) the growth condition (slope or plane) of the plant; (2) the specific orientation (up- or down-slope) of the lateral root tested.

The ''self-loading'' force does not remain constant during the life of the plant, and the reason for this may be found in the continuous shoot meristematic activity that increases the weight of the aerial part. It is obvious then that if the aerial part increases its biomass, the amount of ''self-loading'' forces and/or the direction of force distribution to be dissipated by the roots increases. To stand up and avoid overturning, the root system must become stronger and probably, this adjustment is slightly in advance of the force increase (Ennos 1994). This consideration raises the need to analyze the response of the root system to the ''self-loading'' forces during plant development. To accomplish this, we constructed an artificial slope in a nursery where *Quercus cerris* seeds were sown and seedlings were excavated at different developmental stages. After 2 years of growth the root system of this species showed a complete absence of lateral roots, which could play any mechanical function. Therefore, during the first years of growth, the only mechanically active element present in the root system with a anchoring action is the taproot. In these plants, the ''self-loading'' forces start to be distributed onto lateral roots at a later stage of development, when the mechanical anchorage function of the taproot becomes insufficient.

The picture emerging from our investigations is that, in plants growing on a slope and provided with a rod-like taproot, the initial self-loading forces are distributed to the ground via the taproot. This mechanical action of the taproot is replaced, at a later stage, by the action of the lateral roots (in particular, the structural first-order laterals as suggested below) which likely act as stabilizing guy ropes in the up- and down-slope direction. Despite the fact that we have accumulated sufficient data to indicate that plants growing on a slope have an asymmetric distribution in response to ''self-loading'' forces, we still lack information on whether or not the lateral roots growing in the two directions (up- and down-slope) are normally in tension or in compression. Literature reports that, in the case of mechanical stimulus generated by the wind, the roots growing in the leeward direction are put into compression and those growing in the windward direction are put in tension (Coutts 1983a,b; Barlow 1994; Stokes and others 1997; Stokes and Guitard 1997). These observations in the field have been confirmed by similar observations in the nursery where the young Sitka spruce and the European larch (*Larix deciduas* P. Mill), grown in wind tunnels, showed that wind stress resulted in an uneven development of lateral roots around the plant's central axis, with thicker roots along the axis of the wind direction. These changes induced an increase of the surface area on the windward side with the result of improving the mechanical stability of the plant (Stokes and others 1997) by better transferring the tension to soil via friction (Stokes and others 1996). In analogy with what is known about the wind stimulus, we predict that in plants growing on a slope, the ''self-loading'' forces distributed in lateral roots induce a tension strength in the up-slope growing roots, and a compression strength in the down-slope growing roots. In our case, the roots being under tension strength tend to be elongated whereas those being under compression strength tend to be shortened.

SPECIFIC ROOT SYSTEM ARCHITECTURE INDUCED BY SLOPE

The importance of root system architecture for the stability of trees has received considerable attention (Coutts 1983a,b, 1986; Crook and Ennos 1996; Goodman and Ennos 1999; Nicoll and Ray 1996; Stokes and others 1996, 1997). One work, dealing with the root system of plants growing on a slope, suggests that the response to the overturning forces is based upon the development of an asymmetrical root system to more efficiently distribute the tensile strength among lateral roots (Watson and others 1999). However, an unequivocal correspondence between slope and the deployment of an asymmetric root system has not yet been demonstrated.

With the aim of investigating whether the anchorage biomechanics of plants growing on a slope is based upon the deployment of a specific root system architecture, we compared the root system architecture of five different woody plant species, the trees *Quercus pubescens*, *Quercus cerris*, *Fraxinus ornus*, the shrub *Spartium junceum*, and the herbaceous plant *Cardopatum corymbosum*. These species present root systems with considerable morphological differences and therefore it is reasonable to hypothesize that they differ considerably in the biomechanical distribution of ''self-loading'' forces. Despite the occurrence of specific morphological root system differences, all five woody species investigated present an on-slope asymmetrical architecture, which we call ''*bilateral fan-shape*'' (Figure 2). This architecture differs considerably from the one we called ''*symmetrical bell-shape*'' which is characteristic of the same species growing on a plane (Chiantante and others 2003). The bilateral fan-shape architecture results from lateral roots growing mostly in the up- or down-slope direction whereas the symmetrical bell-shape architecture results from lateral roots growing symmetrically
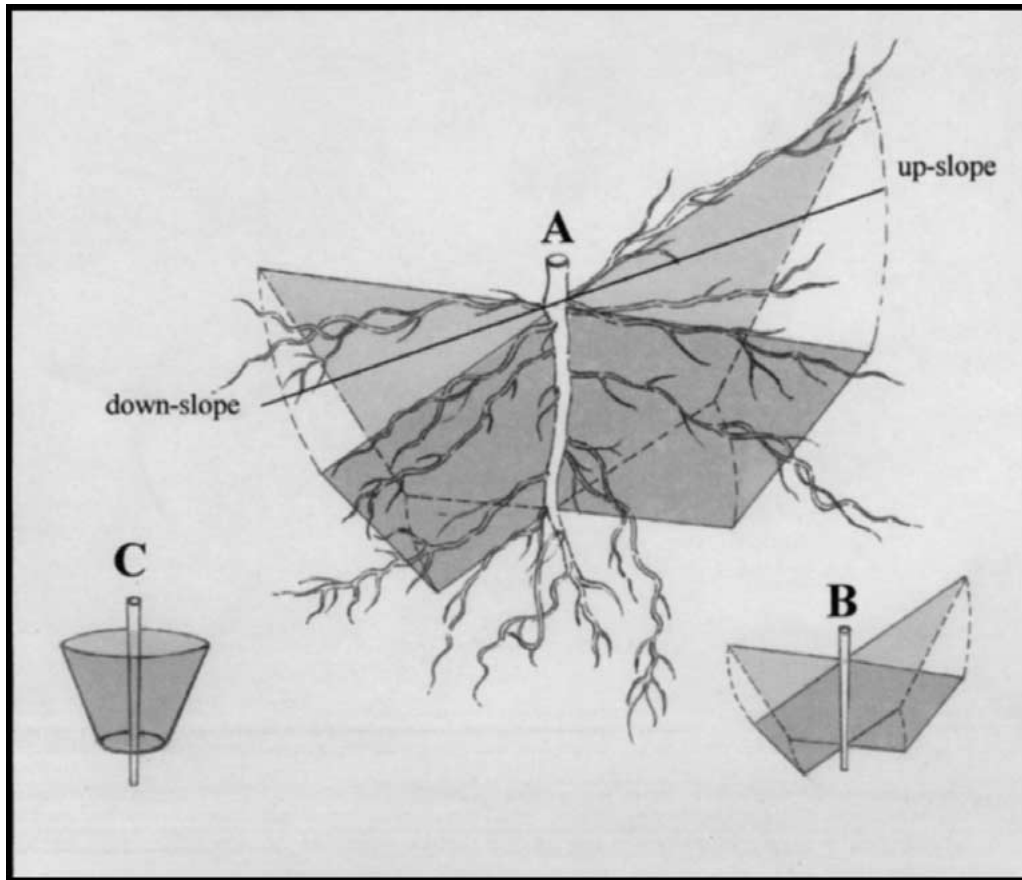
Figure 2. Distribution of first-order laterals of a *Fraxinus ornus* seedling within a *"bilateral fan-shape"*. The distribution of first order laterals when the seedlings grow on a slope is represented in the drawing. The bilateral-fan-shape (**A**) is formed by two sectors up-slope or down slope (**B**). When seedlings grow on a plane the first order laterals form a symmetrical bell-shape (**C**).

around the taproot (Chiatante and others 2003). Therefore, based on our observations of diverse plant species, it seems reasonable to suggest that when a plant grows on a slope its root architecture assumes a bilateral fan-shape disposition.

When is a plant able to perceive the slope and to develop a bilateral fan-shape disposition? To answer this question and to understand when the bilateral fan-shape architecture develops, we investigated the development of the root of *Spartium junceum* seedlings at different times (11, 22, 30, 60, 90, 120, and 160 days) after the beginning of germination. The excavated taproot was divided into 1-cm lengths starting from the root collar and proceeding in the direction of the apex. The number of first-order laterals per unit is recorded together with their respective growth direction. The data show that the emergence of lateral roots starts in the more distal units of the taproot, in the region of the collar, and proceeds toward the root apex contemporaneously with taproot elongation. This emergence well re-

flects the random stochastic model observed by other authors for different plants (Jourdan and Rey 1997; Dubrovsky and others 2000). From the point of view of root system architecture, the seedlings grown on a slope in our glasshouse experiment 160 days from germination formed an asymmetric root architecture with few lateral roots distributed mainly along the up- or down-slope direction. In contrast, seedlings grown on a plane showed a more symmetrical root development of lateral roots around the taproot. The length of the taproot, the rate of lateral elongation along the taproot, and the total number of laterals remained the same in both conditions: on a plane and on a slope. However, at such an earlier stage of development when few lateral roots are present, it becomes impossible to consider this asymmetric architecture as belonging to the bilateral fan-shape type. Nevertheless, we consider this initial asymmetrical root system as the first step toward the construction of the definitive architecture that will characterize adult plants living on a slope.
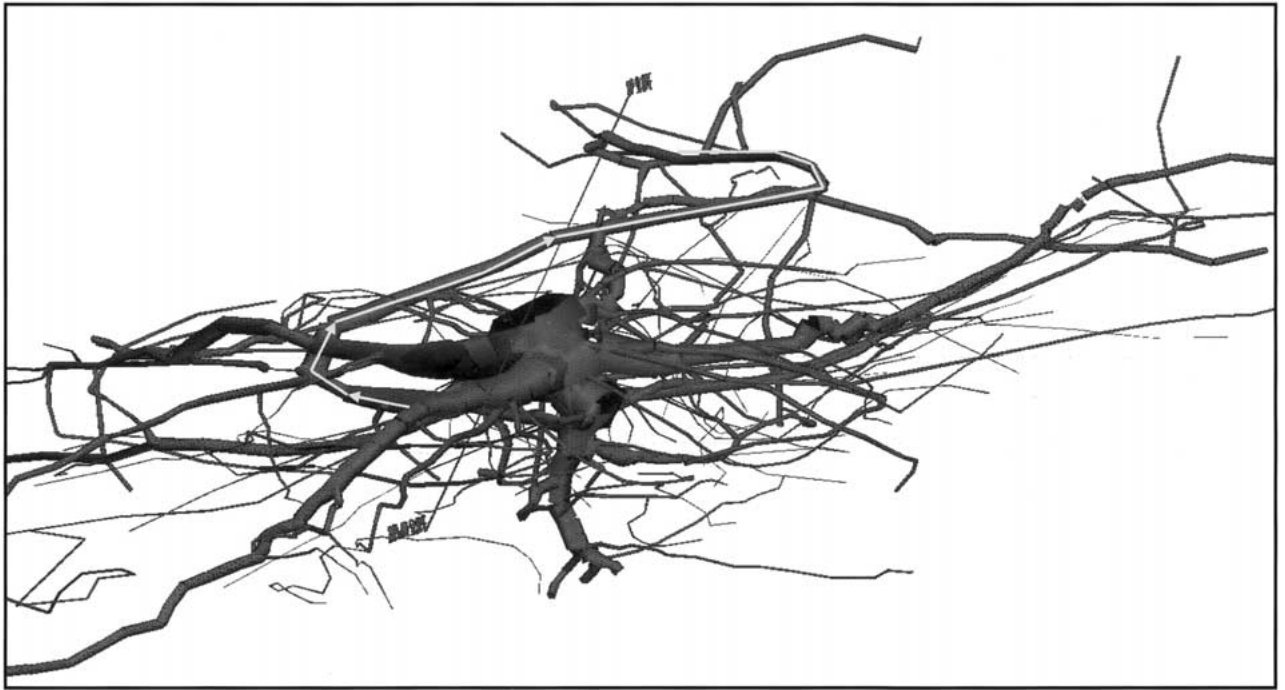
Figure 3. Root architecture of *Quercus cerris* with a lateral root showing a change in growth direction. The figure shows that a lateral root (arrows), which has been emitted in the down-slope direction, turns around the taproot and grows in the up-slope direction, becoming one of the longest laterals present in the root system.

We have frequently observed an anomaly in the bilateral fan-shape architecture of a structural root growing in a direction opposite to that of its initial emergence angle from the taproot. In particular, this structural lateral root emerges with a down-slope orientation but then it turns around the taproot and continues its development in the up-slope direction. This event occurs quite normally when the slope is very steep and it seems to be independent of the species examined (Figure 3). We suggest that this anomalous architecture represents a response of the root system in cases of extreme slope steepness. We believe the change in direction for these roots derives from a superimposition of new stimuli to those received initially at the time of emergence. Support for this hypothesis comes from other studies where it has been shown that the angle of growth to the vertical (the liminal angle) can be modified at a later stage by environmental factors (Coutts and Nicoll 1991).

Finally, we must point out the fact that the bilateral-fan shape architecture of the root system in plants grown on a slope is always associated with a reduction in stem growth. Even at an earlier stage of development, as the one tested in our experimental system (160 days following germination), the seedlings grown on a flat plane have a higher degree of ramification and higher number of leaves than seedlings grown on a slope. These data, obtained in the glasshouse, are confirmed by our observations in the field where a difference in branching and dimension of the aerial part is visible with lower values for plants grown on a slope. We have not yet finished examining these differences in our plants, but they seem to be similar to those reported in the literature for responses to other mechanical stresses such as wind and with other species (Stokes and others 1995; Hepworth and Vincent 1999). However, the differences found in the stem of seedlings grown on a plane or on a slope in our studies contrast strikingly with the general similarity of root traits examined (taproot length, and number of laterals per taproot unit). This contrast is more puzzling when we consider that seedlings with a lower self-loading weight, seedlings growing on a slope, have the same taproot length and the same number of lateral roots as those growing on a plane and characterized by a higher self-loading force. Hence, even at an earlier stage of development, there seems to be a necessity for a more branched taproot for seedlings growing on a slope even in the presence of a lower self-loading force. The considerable differences found in the root system at a later stage of development could be explained by the hypothesis that in adult plants the anchorage function is probably entrusted preferentially upon a
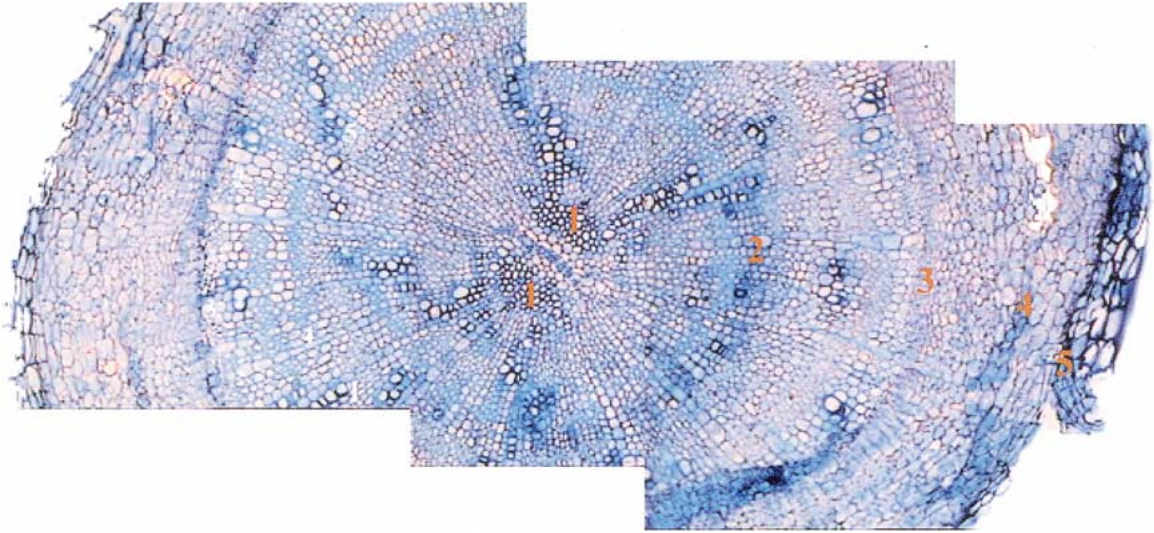
Figure 4. Secondary xylem structure in *Spartium junceum* taproot. Cross-section cut at the level of the root collar shows that the pericycle is missing and development of a secondary xylem structure is evident (1. Primary xylem; 2. Secondary xylem; 3. Cambium; 4. Secondary phloem; 5. Cork).
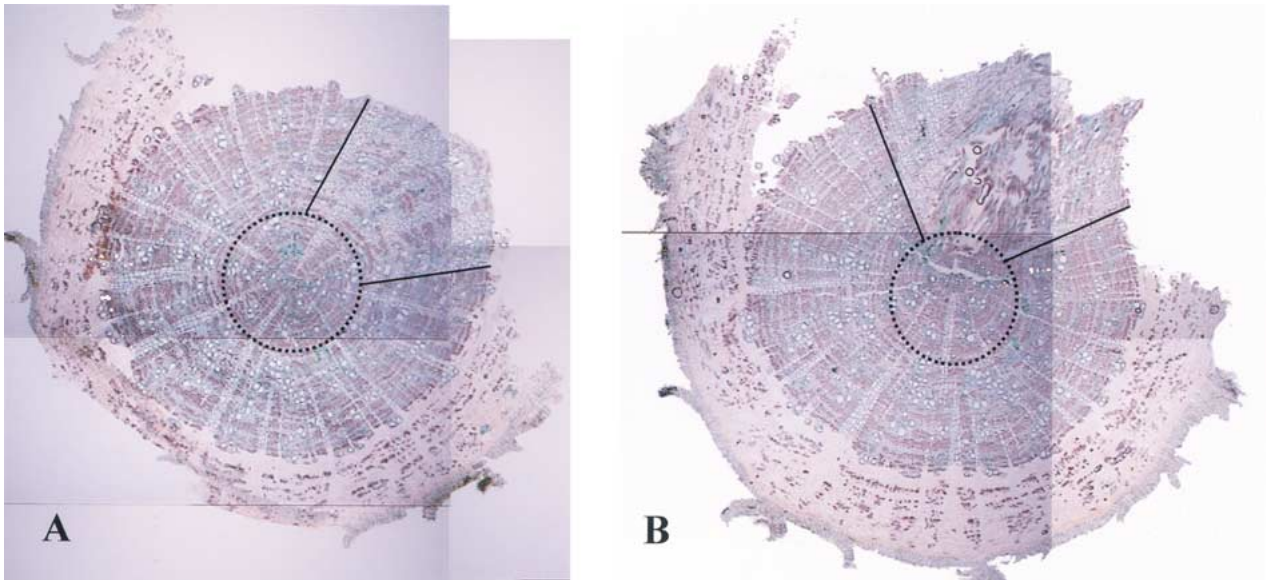

Figure 6. Emergence of lateral roots in woody root apparatus. The parental root is cut before the branching point (**A**) or at the level of the branching point (**B**). The dotted line includes the first portion of secondary xylem where new rays are visible. Externally to the dotted line there is another portion of secondary xylem showing considerable concentration of rays in a particular direction (within the two lines). In (**B**) the connection between the xylem of the lateral root and the xylem of the parental root is visible. It is possible that the formation of a new lateral primordia starts at the boundary of the dotted line with the contribution of a number of rays.

limited number of more powerful structural roots characterized by specific growth directions. As a consequence, the overall number of roots present in the root system of plants growing on a slope may be lower than that of plants growing on a plane, as reported above.

LATERAL ROOT EMERGENCE

Lateral root emergence is an aspect of root biology that has remained poorly understood for a long time (Charlton 1991; Fitter 1991), despite the fact that it had been earlier proposed that the growth direction
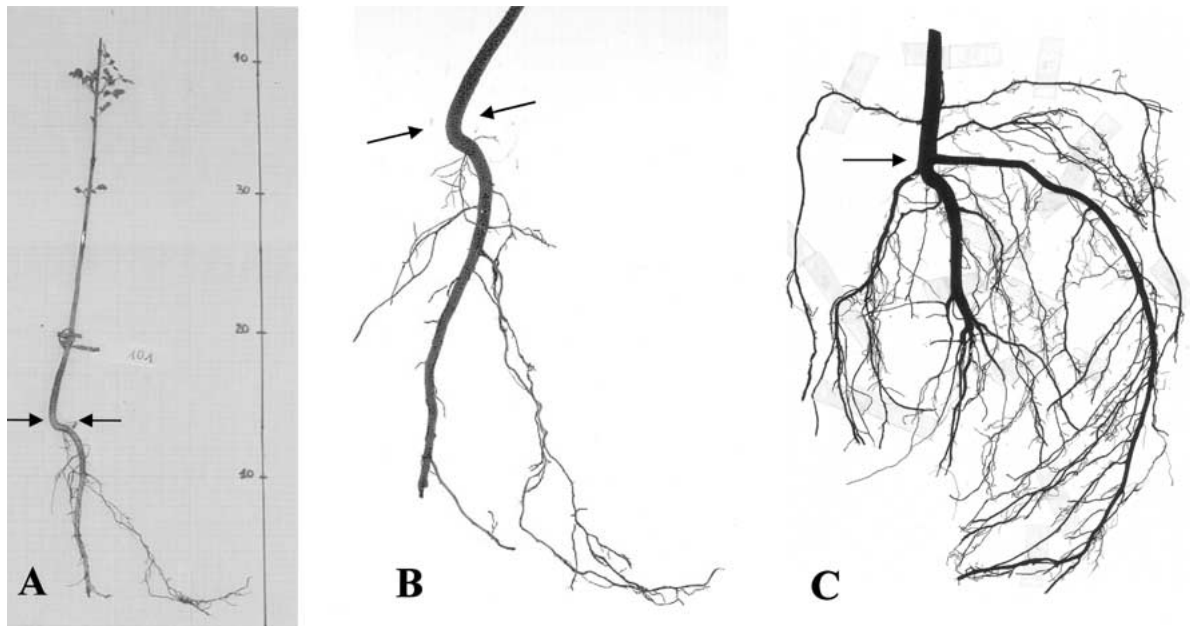
Figure 5. Emission of a lateral root from a woody portion of a taproot in *Fraxinus ornus*. The figure shows the root system in a seedling (**A**, **B**) before its transplantation in the nursery bed. After one year of growth, the same seedling (**C**) has been excavated and the root system shows that a considerable number of lateral roots have emerged. The new lateral roots with a larger diameter are those emitted from the root collar (see arrows in B and C) where a secondary woody structure is present.

of laterals could be determined at an early stage (Champagnat and others 1974). More recent evidence indicates that lateral root formation is specified within the root apical meristem (Dubrovsky and others 2000), probably in advance of visible initiation of the primordium (Hinchee and Rost 1992). The differentiation of competent pericycle cells (Gladish and Rost 1993; Malamy and Benfey 1997a, b) seems to be the starting event for lateral root primordium initiation. These pericycle competent cells form columns of cells distributed in the protoxylem radii (Torrey 1986; Peterson and Peterson 1986; Malamy and Benfey 1997a, b; Dubrovsky and others 2000, 2001) from which new lateral roots are formed. Also the biochemical events responsible for pericycle cell recruitment have been recently investigated. In this case, the suggestion emerges that the reentry in the cell cycle from a G2 phase arrest (Blakely and Evans 1979; Malamy and Ryan 2001) is marked by expression of cyclin and cdc2 genes (Hemerly and others 1993; Doerner, 1994; Ferreira and others 1994; Martinez 1992; Beckman and others 2001). These events seem to be under auxin control (Kerk and others 2000). Interesting observations, for their implications regarding the induction of lateral root primordia under a mechanical signal are studies relating mechanical stimulation to expression of specific

genes such as HRGP containing an extensin domain (Keller and Lamb 1989). In particular, the bean HRGP4.1 and the tobacco HRGPnt3 promoter/GUS gene fusion seem to be transiently induced in a subset of pericycle and endodermal cells involved in lateral root initiation (Dolan and others 1993; Wycoff and others 1995).

Support to the hypothesis that changes in root architecture occur after root emergence comes from studies showing that random emergence of lateral roots is influenced by environmental factors. In these cases, root emergence is based on pericycle cell activation (Charleton 1991) and is not restricted to a narrow developmental window (Blakely and others 1982; Laskowski and others 1995; Doerner and others 1996; Dubrovsky and others 2000). Unfortunately, all the studies reported in the literature refer to lateral root emergence from a parental root where the pericycle cells are still present. On the contrary, in our experimental system the new lateral roots continue to be emitted, even at the latest time of our observations (160 days), from the taproot portion near the root collar where a woody secondary body in the distal portion of the taproot is present and pericycle cells are already missing (Figure 4). Therefore, our data raise the question of whether lateral root emergence is still possible from a portion of the taproot where a woody secondary

body has been developed. We call this event ''*late lateral root emergence*'' to distinguish it from the more commonly known case in which emergence of lateral roots refers to the emergence of a lateral root from a parental root with a primary body structure. Mickovski (2001) has suggested that most lateral roots may be emitted from the upper part of the taproot near the trunk base probably by means of a ''late'' root initial cell recruitment (Charleton 1991). It it possible that late lateral root emergence represents the response to the variation of self-loading forces experienced by plants growing on a slope. In fact, an adjustment in the distribution of forces with respect to the ground may result from a change of anchorage function in some lateral roots, or the growth of new laterals with an anchorage function. The possibility of a change in the function of lateral roots has been clearly demonstrated in other works where the authors show that the largest roots in the first few years after sowing and out-planting are not necessarily the largest later on (Coutts and others 1999). In our studies, the reorientation of some structural lateral roots discussed above demonstrates this possible change of function. Nonetheless, no specific study has investigated the occurrence of late lateral root emergence from the taproot (or other woody parental root) during the entire life of a plant.

To shed some light on this hypothesis, we undertook a simple experiment that consisted of excavating one-year old saplings from a plane nursery bed; we then scanned the root system of excavated saplings by means of computerized image analysis software (WinRhyzo by Regent Ltd, Canada). The saplings were transplanted in a field nursery and re-grown for one year on a plane or on a steep slope. The saplings were re-excavated and their root systems were scanned again in search of new lateral roots that grew from the woody portion of the taproot. The species that we used in this experiment were *Quercus cerris*, *Quercus pubescens*, *Fraxinus ornus*, and *Spartium junceum*. All the species tested were able to emit new first-order laterals after transplantation even in the most distal woody portion of the taproot where a woody structure was certainly present. Interestingly, the most distal new lateral roots, in the case of transplantation on an artificial-slope bed in the nursery, were always emitted in an up-slope direction (Figure 5). At present, we are still investigating this event (unpublished) from an anatomical point of view by means of serial cross-sections of the woodcut at the level of the branching point.

Certainly the hypothesis of late lateral root emergence for anchorage reinforcement would find support in the demonstration that a lateral root primordium may be formed also by the recruitment of initials different from pericycle cells. But unfortunately, the research in this field is missing even though an interesting suggestion comes from the existing literature about adventitious root emission from the stem (Kozlowski and others 1991). In fact, it is known that adventitious roots might be obtained from the recruitment of parenchyma cells from the medullar rays present in the phloem (Lovel and White 1986). We have not yet investigated the first phases of primordium initiation in a portion of parental root characterized by a secondary body structure. However, when a cross section of a lateral branching point was examined, we found that several medullar rays of the parental root were in the direct vicinity of a lateral root. Our anatomical investigations suggest that a mechanism starting with the onset of cell division in the cambium cells and/or parenchyma cells in the ray zone of the secondary phloem, could be responsible for the late lateral root emergence observed in our experiments. According to this hypothesis, this event would represent an emergency response of the root system to specific mechanical stimuli that require anchorage improvement. Probably the number and orientation of rays involved in this event could be a determinant for orientation and shape, which will characterize the new lateral root under construction (Figure 6).

If the occurrence of late lateral root emergence is confirmed, the ultimate architecture (''bilateral-fan shape'') of a root system in an adult plant living on a slope would result from two consecutive events. The first event involves the emergence of lateral roots according to a predetermined (genetic) program established in the meristematic apex of each parental root. The second event includes the modification of the mechanical function of some lateral roots, and the late emergence of lateral roots. The addition of this second event upon the first would be responsible for the deployment of architecture in the root system, which responds better to the anchorage needs of plants growing on a slope.

MODIFICATION OF ROOT DIAMETER, SHAPE, BRANCHING, AND STIFFNESS

It is accepted in the literature that not only are root systems able to respond locally to mechanical stimulation, but that this leads to a modification of root traits that improve anchorage strength (Goodman and Ennos 1996, 1997, 1999). For example, in
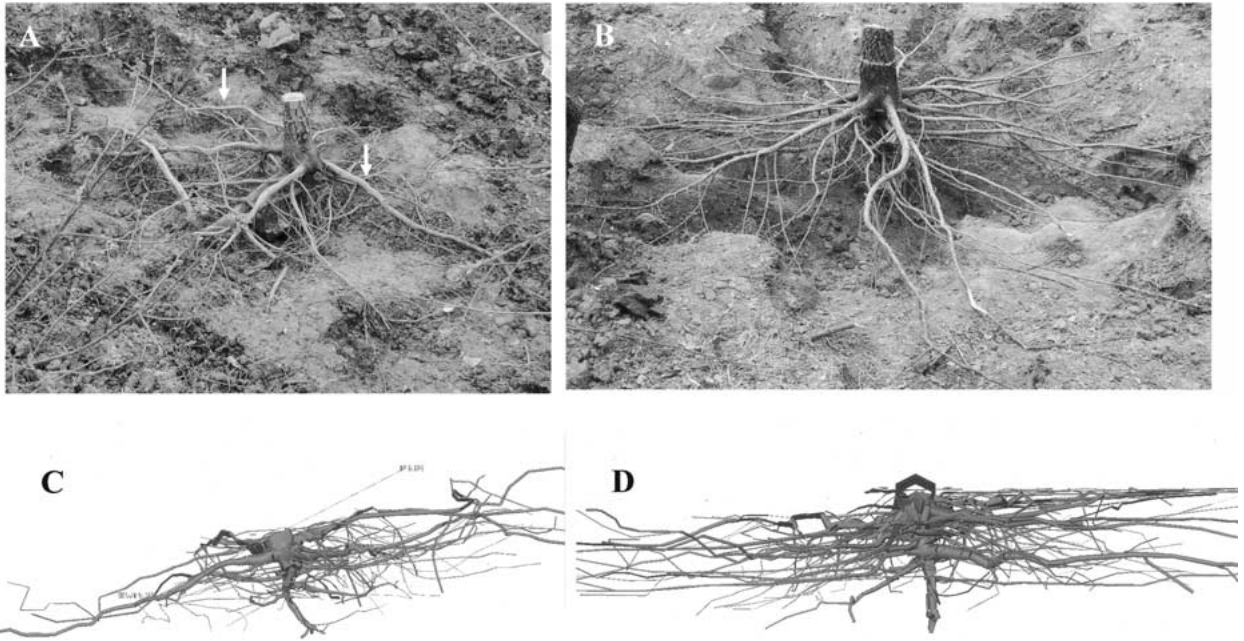
Figure 7. Root system of *Quercus cerris* growing on a slope (**A**) or on a plane (**B**). The two root systems were hand excavated and then taken into the laboratory and placed on a stand in their original orientation. The growth direction of each lateral root has been reconstructed in digital form with the Polemus 3D digitizer. (**C**) The computerized image of the root system shown in (**A**). (**D**) The computerized image of the root systems shown in (**B**).

the case of unidirectional wind stimulation (Fitter and Ennos 1989), where the mechanical stimulus is not symmetrically distributed on the root system, a close association of root system resistance moment with root number has been observed (Duparque and Pellerin 1994; Stokes and others 1996; Guigo and Herbet 1997), as well as changes in root angle (Crook and Ennos 1994) and root diameter (Herbert and others 1992). The response to wind mechanical stimulus seems to involve the growth of a stiffer ''taproot'' which increases its secondary thickening, and an increase in one or several lateral root traits such as diameter. This type of response to mechanical stimulation has been observed in both herbaceous plant species (Goodman and Ennos 1996, 1997) and trees (Stokes and others 1997), although different species responded in different ways with dicots showing less effect than monocots (Materechera and others 1991).

In analogy with studies on wind effect, we are interested in what kinds of traits are modified in the root system of plants growing on a slope in order to reinforce plant anchorage. Obviously, the chemical and physical properties of the soil may have a great influence upon root growth because they affect the distribution of forces from the roots to the ground. In fact, it has been suggested that any consideration regarding plant anchorage should evaluate both the properties of the root system and of the soil (Ennos

1994; Pagés 2001). For this reason, we have examined soil properties in the sites of our excavations. The results of these analyses show a complete similarity among all the soil properties found in the various sites examined. Therefore, we don't believe the differences in root traits observed between plants growing on a slope and plane are caused by differences in soil properties.

After excluding the interference of soil, we focussed on the variation of the diameter, the shape, the branching, and the stiffness of the root system. At present, these traits have been investigated, particularly in the structural roots, which may be defined as those lateral roots having a diameter greater than 2.0 cm at a distance of 20 cm from the tree trunk (Mickovski 2001). In our studies, the trees (25–30 years old) growing on a slope always showed 3–4 structural roots with a very large diameter (6–8 cm) at a distance of 20 cm from the tree trunk, and always emerged from the first 20 cm of the taproot length. These results contrast considerably with those obtained from trees of the same age grown on a plane. Plane-grown trees rarely present lateral roots with a very large diameter at the same distance from the trunk as those observed on a slope. A general conclusion from field observation is that an optimal anchorage system for plants growing on a plane should contain an intermediate number of 10–100 roots in addition to an adequate
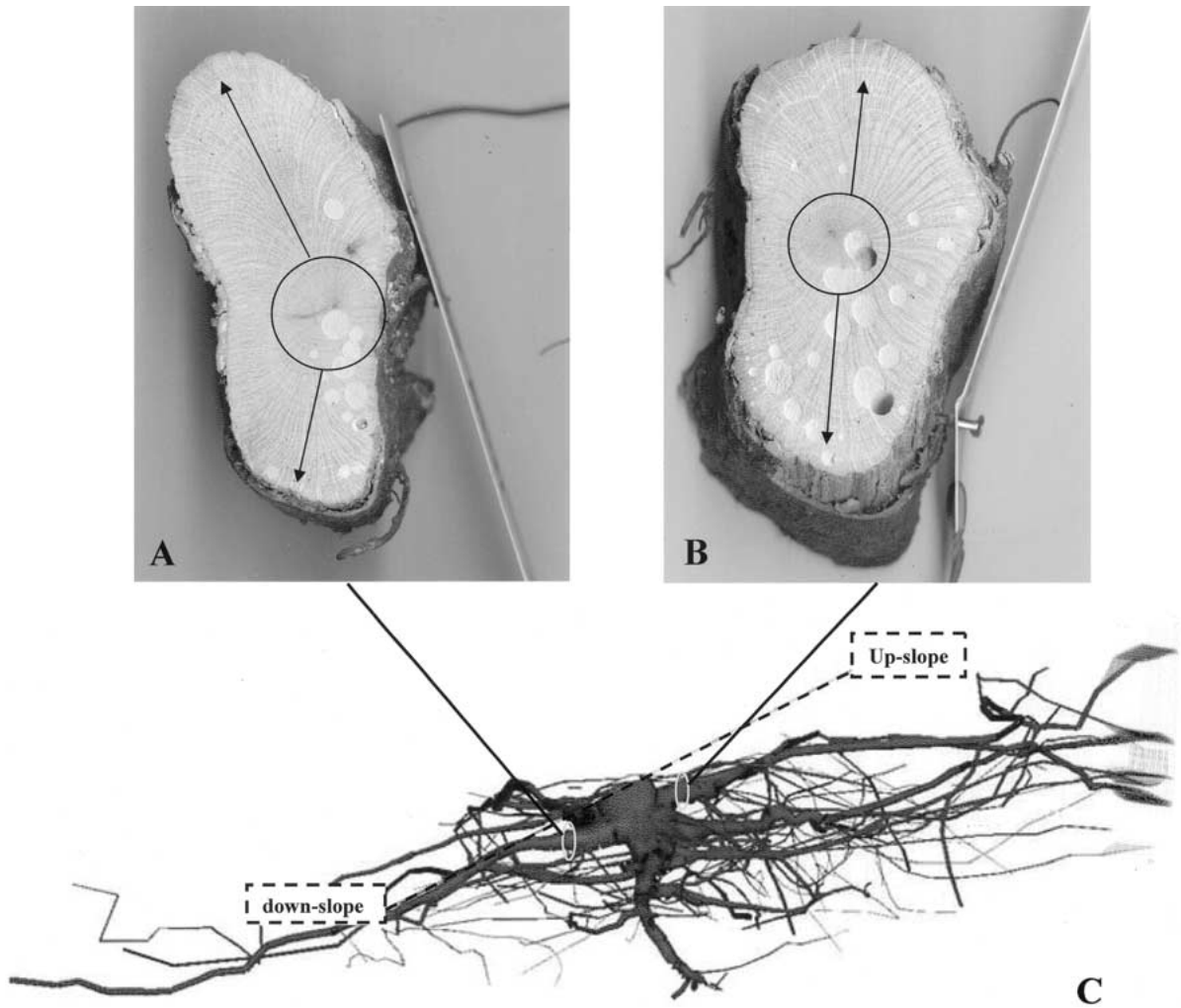
Figure 8. Eccentricity of the up-slope and down-slope lateral roots in *Quercus cerris*. The two woody sections shown (**A**, **B**) have been cut at a distance of 20 cm from lateral root bases (**C**). The circles indicate the portion of wood characterized by a visible circular symmetry. The arrows indicate the directions in which wood has been added probably in response to the mechanical stimulus. The amount of wood in the down-slope lateral (**A**) is not equally distributed, unlike the up-slope lateral roots (**B**).

taproot (Ennos 1994). Higher numbers of roots would cause soil adhesion to fail, and allow the roots and surrounding soil to be pulled intact from the ground with little force (Ennos 1994). In our investigations, we observed that the overall number of roots in the root system is higher on a plane than on a slope (Figure 7), but we have not identified a critical number of roots for plants growing on a slope that could be related in someway to anchorage failure.

Stokes and Guitard (1997) found in woody roots that when the amount of stress perceived by the cambium differs at particular points around its circumference, the response gives rise to tension wood. In fact, these authors suggest that the shape of the root system may be used as an indicator of how mechanical forces are distributed along the root system. Nicoll and Ray (1996) reported that roots often have greater thickening on their upper sides, producing a shape comparable to a ''T-beam'' close to the tree base. Farther from the tree, many roots develop cross-sectional shapes comparable to ''I-beams'' that resist vertical flexing better than any other shape with the same cross-sectional area rectangular, elliptical, or circular (Nicoll 2000). We have noticed that in the root system of plants growing on a slope, the structural roots also present a considerable tension wood eccentricity at their bases (Figure 8). The eccentricity in the root wood is present in up- or down-slope structural roots and decreases at a distance of 15–20 cm from the trunk base. Whether the eccentricity observed in our root

systems is the same as that observed in the structural roots with up- or down-slope orientation is still under investigation. In this regard, more work is in progress to analyze growth rings (Richardson 2000) in the wood of both types of lateral roots. In analogy with what has been suggested in the case of the response to mechanical wind stimulus, we propose that the uneven shape found in our structural roots, produces stiffer root systems and reduces the chance of the soil shearing and separating from the roots under self-loading forces (Gardiner and Quine 2000).

The presence of a high incidence of root branching near the stem allows for more rapid dissipation of forces, avoiding a higher investment in strength further along the root (Stokes and Guitard 1997). The mechanical role played by root branching is demonstrated by the fact that mechanically stressed plants presented a higher number of lateral roots than those of the controls (Goodman and Ennos 2001). We have not finished examining the branching of all the root systems of our adult trees (*Quercus cerris* L. and *Quercus pubescens* Willd.); therefore, at present we do not know the role (if any) of root branching in the distribution of forces in plants growing on steep slopes. Previous investigations we conducted on young *Spartium junceum* plants suggested that plants growing on a slope present an increase in the level of root branching (Chiantante and others 2003). Thus, we cannot rule out that branching by an adult woody plant might increase in one (or both) growth directions (up- or down-slope) and improve plant anchorage.

Schiechtl (1980) has shown that roots growing in the uphill direction are stronger than those extending downhill in several tree species. This study suggests that another strategy that improves the anchoring function of a root is an increase in its strength and stiffness by acting upon the properties of the wood itself (Stokes and Guitard 1997; Nicoll 2000; references in Niklas and others 2000). Investigating the wood of lateral roots of *Spartium junceum*, we found that the up-slope lateral roots present a higher percentage of sclerenchyma fibers with respect to down-slope lateral roots. These sclerenchyma fibers appear as though they are not lignified because they did not stain with a lignin staining procedure. At the moment we do not know the mechanical function of the increased number of the fibers in the up-slope growing lateral roots. We have undertaken a comparative analysis of the tensile resistance of up-slope- versus down-slope lateral roots using a tensile strength indicator. The rationale is that up-slope growing lateral roots will show a higher tensile resistance than down-slope growing laterals.

It has been suggested that fluctuations in tensile strength (Hathaway and Penny 1975) may be related to variation of the lignin/cellulose ratio, which is under seasonal and/or abiotic factors, such as mechanical stress (Plomìon and others 2001). In particular, it seems that in tension wood the overall lignin content is lower, the cellulose content is higher, and the microfibril angle is lower than that of corresponding normal wood (Plomìon and others 2001). In cross-sections of lateral roots of *Spartium junceum* growing in the up- and down-slope direction, the lignin staining protocol that we used did not detect differences, but a biochemical measurement of the amount of extractable lignin would be necessary to confirm the absence of differences.

During our studies, we have not investigated variations in the number of cell wall-thickened tracheids as has been suggested by other authors who investigated wood from mechanically perturbed seedlings and trees (Telewski and Jaffe 1986). Nevertheless, in our anatomical investigations we found that the secondary xylem diameter in lateral roots growing in the up-slope direction of seedlings of *Spartium junceum* grown in our glasshouse experimental system is greater than in the down-slope direction. However, the mechanical role (if any) of this difference in secondary xylem diameter remains unknown. The occurrence of different orientations of the cellulose microfibrils in the secondary cell wall of the fibers (Timell 1986; Stokes and Guitard 1997) in up- or down-slope lateral roots is completely unexplored.

CONCLUSIONS

In response to anchorage reinforcement to avoid uprooting, plants growing on a slope develop an asymmetric root system architecture called ''*bilateral-fan shape*''. This architecture seems to derive from a preferential elongation of lateral roots in the two main directions (up-slope and down-slope) and represents the most efficient means of distributing self-loading forces. These up- and down-slope roots are structural roots and present a considerable shape eccentricity at their base, the mechanical importance of which is still under investigation. This bilateral-fan shape architecture might partly result from a change in the mechanical function of existing laterals and partly from new late lateral root emergence from parental roots even if secondary growth is well developed. The great interest for this second hypothesis arises from the fact that lateral

roots emerging from secondary growth would enable a plant growing on a slope to continuously adjust its anchorage function of self-loading forces variations. A question that needs to be answered is whether or not it is possible to speak of an adaptation to slope conditions. This would be at the basis of the search for an ecotype better adapted to slope conditions.

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