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Trade-off between root porosity and mechanical strength in species with different types of aerenchyma

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ABSTRACT

The objective of this work was to study the existence of a trade-off between aerenchyma formation and root mechanical strength. To this end, relationships among root anatomical traits and mechanical properties were analysed in plant species with contrasting root structural types: *Paspalidium geminatum* (graminaceous type), *Cyperus eragrostis* (cyperaceous type), *Rumex crispus* (*Rumex* type) and *Plantago lanceolata* (*Apium* type). Variations in anatomical traits and mechanical strength were assessed as a function of root diameter by exposing plants to 0, 7, 15 and 30 d of control and flooded conditions. For each species, the proportion of root cortex was positively associated with the increment of root diameter, contributing to the increase in root porosity under both control and flooded conditions. Moreover, cell lysis produced an additional increase in root porosity in most species under flooded conditions (except *R. crispus*). Both structural types that presented a uniseriate layer (epidermis) to cope with compression (*Rumex* and *Apium* types) were progressively weakened as root porosity increased. This effect was significant even when the increment of root porosity was solely because of increased root diameter (*R. crispus*), as when both processes (root diameter and cell lysis) added porosity to the roots (*P. lanceolata*). Conversely, structural types that presented a multiseriate ring of cells in the outer cortex (graminaceous and cyperaceous types) maintained mechanical strength over the whole range of porosity, in spite of lysogenic processes registered in the inner cortex. In conclusion, our study demonstrates a strong trade-off between aerenchyma formation and mechanical strength in root structural types that lacked a multiseriate ring of tissue for mechanical protection in the outer cortex. The results suggest that this ring of tissue plays a significant role in maintaining the mechanical strength of roots when flooding induces the generation of additional aerenchyma tissue in the root cortex.

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Key-words: *Cyperus eragrostis*; *Paspalidium geminatum*; *Plantago lanceolata*; *Rumex crispus*; aerenchyma arrangement; flooding; root diameter; root strength; soil compaction.

INTRODUCTION

Plants inhabiting periodically flooded environments have to deal with oxygen shortage as a major stress factor. To cope with this stress, flood-tolerant species usually develop anatomical and morphological changes related to their survival under anaerobic conditions (Armstrong 1979; Voesenek & Blom 1989). The most studied plastic response to flooding is the formation of aerenchyma in the root cortex (Smirnoff & Crawford 1983; Justin & Armstrong 1987; Colmer *et al*. 1998; Visser *et al*. 2000; McDonald, Galwey & Colmer 2002; Colmer 2003; Grimoldi *et al*. 2005; Visser & Bögemann 2006). A system of interconnected aerial spaces (aerenchyma) provides a pathway of lower resistance that facilitates oxygen transport from aerial shoots to the roots, allowing root growth and soil exploration under anaerobic conditions (Armstrong 1979; Colmer & Greenway 2005). However, the formation of additional aerenchyma in the root cortex produces large variations in the internal structure of the roots (Justin & Armstrong 1987), which might lead to a trade-off in root mechanical strength necessary to resist both natural and anthropogenic soil compressive forces (Engelaar, Jacobs & Blom 1993a; Striker *et al*. 2006).

The aerenchyma arrangement in the root cortex in response to flooding is variable among species (Smirnoff & Crawford 1983; Justin & Armstrong 1987; Visser *et al*. 2000; McDonald *et al*. 2002; Grimoldi *et al*. 2005). Four root structural types (graminaceous, cyperaceous, *Apium* and *Rumex*) have been described based on the spatial arrangement of the aerenchyma tissue and the packing of the cells in the cortex. The basic structures of these root types resemble a bicycle wheel (graminaceous), a spider web (cyperaceous), a honeycomb (*Rumex*) and a non-organized structure with irregular aerenchyma lacunae (*Apium*) (Justin & Armstrong 1987; Seago *et al*. 2005). Variations in root aerenchyma induced by flooding have been investigated regarding the effects on internal root aeration and

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radial oxygen loss (ROL) from the plant to the soil (Schuette, Klug & Klomparens 1994; Colmer *et al*. 1998; Visser *et al*. 2000; McDonald *et al*. 2002; Colmer 2003). Interestingly, these studies reported a much higher ROL in *Rumex* and *Apium* in comparison with graminaceous and cyperaceous root structural types. In these latter types, a multiseriate ring of densely packed cells in the outer cortex appeared to prevent oxygen loss to the rhizosphere (Visser *et al*. 2000; McDonald *et al*. 2002). However, the mechanical response to compressive forces in the main root structural types, influenced by their contrasting aerenchyma arrangements, has never been addressed.

Roots commonly suffer mechanical stress during their lifespan (Bennie 1996). Soil swelling–shrinkage as a result of repeated wetting–drying cycles and animal trampling are major factors producing mechanical stress on roots in grassland ecosystems. Depending on the soil type and condition, such stresses can involve pressure ranging from 120 to 200 kPa because of soil shrinkage (Richards & Greacen 1986; Clark *et al*. 1996; Kirby & Bengough 2002; Bengough *et al*. 2006) and close to 300 kPa because of cattle trampling (Scholefield & Hall 1986). Both types of mechanical stress can lead roots to collapse, therefore hindering water and nutrient uptake (Bengough *et al*. 2006). Furthermore, in many grassland regions of the world, root systems could be exposed to the combination of antagonistic stress factors such as flooding and soil compaction as part of the natural disturbance regime (Soriano 1992). In such a context, the advantage of increased root porosity for oxygenation could endanger the mechanical strength of the roots, which helps them resist trampling (Engelaar *et al*. 1993a,b; Striker *et al*. 2006), and the subsequent soil shrinkage associated with the decrease of soil water content immediately after flooding (Taboada *et al*. 2001). We recently found that the graminaceous root structural type of *Paspalum dilatatum* Poir. does not lose mechanical resistance as root porosity increases in response to flooding, while the *Apium* root structural type of *Lotus glaber* Mill. was weakened twofold by the increment of root porosity (Striker *et al*. 2006). We now extend the analysis to examine the anatomical traits and mechanisms that give roots protection from collapse due to compressive forces.

The strength of vegetal organs, such as roots, depends on its structural type, size and constitutive materials (Niklas 1992; Aranwela, Sanson & Read 1999). The resistance of the organ is expected to be higher if there is a presence of strong mechanical tissues beneath the epidermis, for example, the reported sclerenchymatous ring in rice stems (Li *et al*. 2003). Moreover, anatomical and mechanical properties are known to change in conjunction with the size of the organ (Niklas 1992; Aranwela *et al*. 1999), and flooding is known to directly alter root dimensions (Visser *et al*. 2000). It was reported that flooding could induce the increment of root diameter during the processes of cell division, expansion, separation and lysis that precede aerenchyma formation in many species (Justin & Armstrong 1987; Visser *et al*. 2000; Grimoldi *et al*. 2005; Seago *et al*. 2005). For this reason, we took into account possible size-scaling effects by studying anatomical and mechanical properties as a function of root diameter. This distinction is important for disentangling the mechanisms involved in the formation of additional aerenchyma and its effects on root mechanical strength. Accordingly, significant size effects were found to modify tensile strength (Genet *et al*. 2005), oxygenation efficiency (Laan *et al*. 1991) and the hydraulic conductivity (Rieger & Litvin 1999) of root systems.

The specific objectives of this study were to: (1) investigate the effects of aerenchyma formation on the mechanical strength of roots; (2) analyse the anatomical features associated with mechanical strength properties; and (3) evaluate the extent to which the mechanical properties of roots scale with root size. For this purpose, variations in anatomical traits and mechanical properties were quantified as a function of root diameter by exposing plants to both control and flooded conditions. Our analyses involved four plant species that represent the principal root structural types described by the cited literature: graminaceous, cyperaceous, *Rumex* and *Apium* types. To our knowledge, this is the first mechanistic study on the relationship between root porosity and mechanical strength. We also provide the first quantitative analyses of the scaling-size effects of anatomical and mechanical root parameters.

MATERIALS AND METHODS

Plant material and experimental design

A controlled experiment was carried out with individuals of four species representing the principal root structural types (*sensu* Justin & Armstrong 1987; Seago *et al*. 2005): *Paspalidium geminatum* (Forssk.) Stapf (graminaceous type), *Cyperus eragrostis* Lam. (cyperaceous type), *Rumex crispus* L. (*Rumex* type) and *Plantago lanceolata* L. (*Apium* type). All species are perennials and coexist in plant communities located in the lowland areas of the Flooding Pampa Grasslands in Argentina (Soriano 1992). These grasslands are subjected to continuous cattle grazing (trampling stress) and periodical exposure to flooding of varying intensities and duration (Insausti, Chaneton & Soriano 1999). Selection of the target species was based on preliminary anatomical observations (Justin & Armstrong 1987; Laan *et al*. 1989; Grimoldi *et al*. 2005), involving contrasting root structures as a consequence of the spatial arrangement of aerenchyma tissue and the presence or absence of a multiseriate ring of cells in the outer layers of the cortex (Table 1).

Plants of similar size of the target species were extracted in soil blocks ($0.2 \times 0.2 \times 0.15$ m depth) from an extensive stand of lowland grassland located in the Department of Pila, Province of Buenos Aires, Argentina (36° 30′S, 58° 30′W). Individuals were immediately transplanted into plastic containers (4 L) filled with sand and topsoil from the grassland (1:1), and then transferred to a glasshouse of the Faculty of Agronomy at the University of Buenos Aires. After 6 months of acclimatization, two treatments were randomly applied: (1) control – watered daily to field capacity, and (2) flooded – maintained water level at 4–6 cm

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Table 1. Selected species and their principal root characteristics: structural type[a], aerenchyma formation process, spatial arrangement and cortex features

Species	Aerenchyma		Cortex features
	Formation process	Arrangement	
Paspalidium geminatum Family: Poaceae Root type: graminaceous[a]	Schizo-lysigenous[a,b]	Lacunae radially arranged, bicycle wheel-like appearance	Outer cortex: multiseriate ring of densely packed cells with sclerenchyma Inner cortex: parenchyma with lacunae, and endodermis
Cyperus eragrostis Family: Cyperaceae Root type: cyperaceous[a]	Tangential-lysigenous[a,b]	Lacunae tangentially arranged, spider web-like appearance	Outer cortex: multiseriate ring of densely packed cells Inner cortex: parenchyma with lacunae, and endodermis
Rumex crispus Family: Polygonaceae Root type: *Rumex*[a]	Expansigenous[b]	Aerenchyma disposed in a honeycomb-like appearance	All cortex: parenchyma with lacunae, and endodermis
Plantago lanceolata Family: Plantaginaceae Root type: *Apium*[a]	Lysigenous[a,b]	Non-organized lacunae, irregularly dispersed in the cortex	All cortex: parenchyma with lacunae, and endodermis

All species presented a uniseriate epidermis.
[a]From Justin & Armstrong (1987).
[b]Seago *et al*. (2005).

above soil surface. A gradient of root porosity was obtained by harvesting plants (6 to 8 per species and harvest date) at 0, 7, 15 and 30 d of flooding. During the experiment, plants were grown under natural photoperiod and the mean temperature of the glasshouse at light period was 19.7 ± 5.3 °C. The flooding treatment was characterized by measuring oxygen diffusion rates (ODR) at a soil depth of 5 cm with platinum microelectrodes (Letey & Stolzy 1964). ODR decreased from 54 ± 2 ($\times 10^{-8}$ g cm^{-2} min^{-1}) to 8 ± 1 ($\times 10^{-8}$ g cm^{-2} min^{-1}) in the first 2 d of treatment and remained near zero in the following days.

Root anatomical measurements

A preliminary test was carried out on flooded plants to evaluate the porosity variation along adventitious roots and to select the position in which to perform compression analyses. We measured fractional porosity in consecutive segments of 2 cm from the apex using a pycnometric technique proposed by Jensen *et al*. (1969), which is based on the weight increase that occurs when root air volume is replaced by water after maceration. For each individual plant, four to five subsamples (ca. 0.25 g fresh weight) of roots were measured. All species reached a plateau of root porosity at the second segment above the apex (Fig. 1). Therefore, we decided to compress roots in segments from 2 to 4 cm above the apex.

During the experiment, measurements of root porosity, mechanical strength (see further discussion) and extraction of root samples from each species and treatment were simultaneously done at each harvest. Samples were washed and immediately fixed in alcohol 70% until needed. Root samples of approximately 0.5 cm length between 3.2 and 3.7 cm above the apex were dehydrated in an ethanol series and embedded in paraffin. Cross sections of 7–8 μm thick were cut with a rotatory microtome and double stained

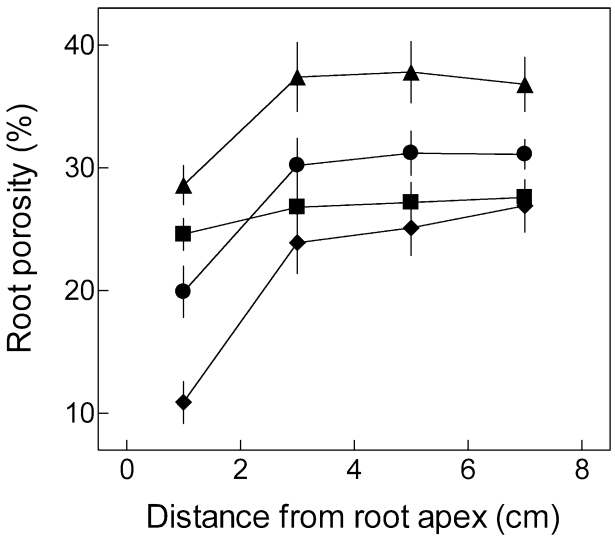

Figure 1. Porosity along adventitious roots of *Paspalidium geminatum* (▲), *Cyperus eragrostis* (●), *Rumex crispus* (■) and *Plantago lanceolata* (◆) plants grown for 30 d under flooded conditions. Measurements were made on segments cut at 2, 4, 6 and 8 cm above the root apex. Values are means ± SE ($n = 5$).

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with Safranin–Fast Green. Representative images of root sections were taken using an optical microscope (Zeiss Axioplan; Zeiss, Oberkochen, Germany) connected to an image analyser (Imagenation Px; Imagenation Corp., Beaverton, OR, USA). Root diameter and cortex thickness were measured in all cross sections, which allowed us to calculate the proportional contribution of cortex to root diameter. At the end of the experiment, total number of lacunae, mean area of the five bigger-sized lacunae, perimeter of the inner cortex (with aerenchyma) and the area of epidermis plus the outer cortex tissue (without aerenchyma) were measured in all species (four to five roots per plant in five plants per species). The ring of cells protecting the inner cortex from collapse was characterized by calculating the ratio between the area of epidermis plus the outer cortex tissue to the inner cortex perimeter. Additionally, in *P. geminatum* and *C. eragrostis*, cell wall thickness along radial and tangential axes of the cells in the outer layers of the cortex were measured (as in Kokubo, Kuraishi & Sakurai 1989). All anatomical measurements were obtained from four to five roots per plant, and by using the public domain ImageTool program (University of Texas Health Science Centre at San Antonio, TX, USA) on digitalized photographs of the cross sections.

Root mechanical strength

We measured the required pressure for root collapse as a quantification of the mechanical strength to radial compression in control and flooded roots (as in Striker *et al*. 2006). Briefly, roots were laterally compressed with a blunt-ended brass shaft (19×11 mm) driven by the piston of a mini-cylinder connected to a pneumatic circuit. A flow regulator (needle valve HOKE 1315G2B; Hoke, Circor Instrumentation Technologies, Spartanburg, SC, USA) connected to the circuit ensured a low constant flux of pressurized air (1.06 L min^{-1} for all measurements) entering to the mini-cylinder. Pressure inside the mini-cylinder increased slowly pushing the piston and compressing the root sample. The circuit was connected to a pressure transducer (ADZ Nagano S-010bar; ADZ Nagano Sensortechnik, Ottendorf, Germany) and a data logger to record the maximum stress that roots could sustain before crush loading (collapse). The compressive strength (kPa) was calculated by dividing the registered force by root compressed area (root diameter × compressed length) (Niklas 1992). Root collapse and root diameter were registered by a binocular loupe with a line reticule in the eyepiece (Wild M5-26388; Wild, Heerbrugg, Switzerland). Measurements were made for segments from 2 to 4 cm above the apex in four to five roots per individual plant. During the measurements, root systems were not separated from the aerial part to avoid turgid loss that could affect the interpretation of the results.

Statistical analyses

Relationships between root porosity and strength within species and the influence of root diameter on root strength, porosity, and cortex proportion were explored by linear regression analyses. When a significant relationship between variables was detected, additional *F*-tests for differences in the regression parameters between control and flooded treatments were performed. If differences between parameters were not detected, data were pooled to obtain a single linear regression. Normality and homoscedasticity of data were checked to satisfy linear regression assumptions. All other anatomical variables were evaluated within species by Student's *t*-test. Variables that involved percentages were ($\arcsin\sqrt{x}$) transformed. All tests were performed using GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego CA, USA).

RESULTS

Effects of root porosity on mechanical strength

A differential effect of root porosity on root mechanical resistance was detected among species with contrasting root structural types (Fig. 2). Under control conditions, the required pressure to cause root collapse was similar in *C. eragrostis*, *R. crispus* and *P. lanceolata* (350–450 kPa), and was twofold higher for *P. geminatum* (800–900 kPa). The root types of *P. geminatum* and *C. eragrostis* maintained the strength of their roots over the entire range of increased porosity ($r^2 = 0.10$, $P > 0.05$ and $r^2 = 0.19$, $P > 0.05$, respectively). In contrast, *R. crispus* and *P. lanceolata* showed a significantly negative relation ($P < 0.01$) between mechanical strength and the increment of root porosity (Fig. 2).

The roots of *P. geminatum* and *C. eragrostis* exhibited a multiseriate ring of tissue surrounding the cortex with aerenchyma, composed by the epidermis and the outer layers of the cortex (Figs 3a–d & 5a,b). In contrast, the roots of *R. crispus* and *P. lanceolata*, which were significantly weakened by the increase of root porosity, presented only a uniseriate layer of cells (epidermis), surrounding the parenchymatic cortex with aerenchyma (Fig. 3e–h). To determine the degree of protection for the aerenchymatic root cortex from external pressure, we calculated the ratio between the tissue area of the ring (epidermis and outer cortex layers) and the inner cortex perimeter. Accordingly, this parameter resulted in an order of magnitude higher in *P. geminatum* and *C. eragrostis* in comparison to *R. crispus* and *P. lanceolata* (Table 2). Moreover, conspicuous differences between *P. geminatum* and *C. eragrostis* were observed: the first species presented three to four layers of densely packed sclerenchymatous cells in the outer cortex, which were not observed in *C. eragrostis* (contrast Figure 5a,c & b,d). Tangential and radial thickness of the cell walls in these layers were 64 and 60% higher in the roots of *P. geminatum* than in *C. eragrostis*, respectively (Fig. 5e,f).

Anatomical traits and mechanical strength as a function of root diameter

Under control conditions, a strong and positive relationship between root porosity and diameter was found in most

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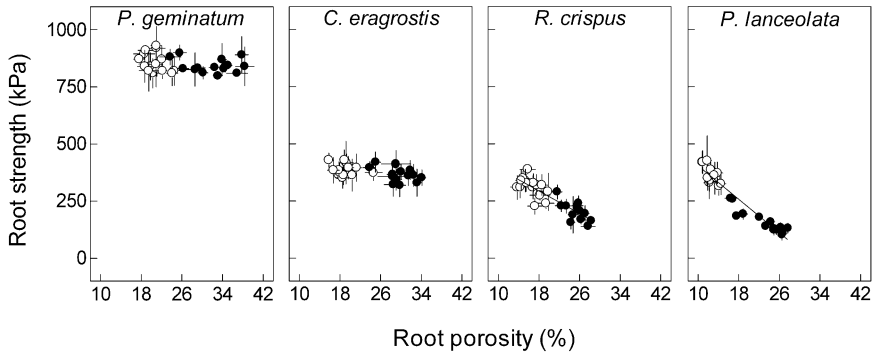

Figure 2. Relationships between root porosity and root strength of *Paspalidium geminatum*, *Cyperus eragrostis*, *Rumex crispus* and *Plantago lanceolata* plants grown under control (○) and flooded (●) conditions. The range of porosity was reached by exposing plants to 0, 7, 15 and 30 d of flooding. Adventitious roots were compressed from 2 to 4 cm above the apex. Solid lines represent significant regressions ($P < 0.001$). When differences between parameters of control and flooded roots were not detected, data were pooled to obtain a single linear regression. Equations are $y = -12x + 520$ (*R. crispus*: $r^2 = 0.77$, $n = 28$) and $y = -18x + 580$ (*P. lanceolata*: $r^2 = 0.91$, $n = 30$). Values represent averages ± SE of four to five roots per plant.

species (Fig. 4). This response was associated with an increase in the proportion of cortex as a function of root diameter (Fig. 4), the section in which constitutive aerenchyma lacunae are located (Fig. 3). Under flooded conditions (besides the intrinsic effect of diameter on root porosity), lysogenic processes largely added porosity to the roots in all species, except *R. crispus* (Fig. 3). Root strength in species that presented a multiseriate ring for mechanical protection (graminaceous and cyperaceous types) were not affected by the increase in root porosity, even as a result of

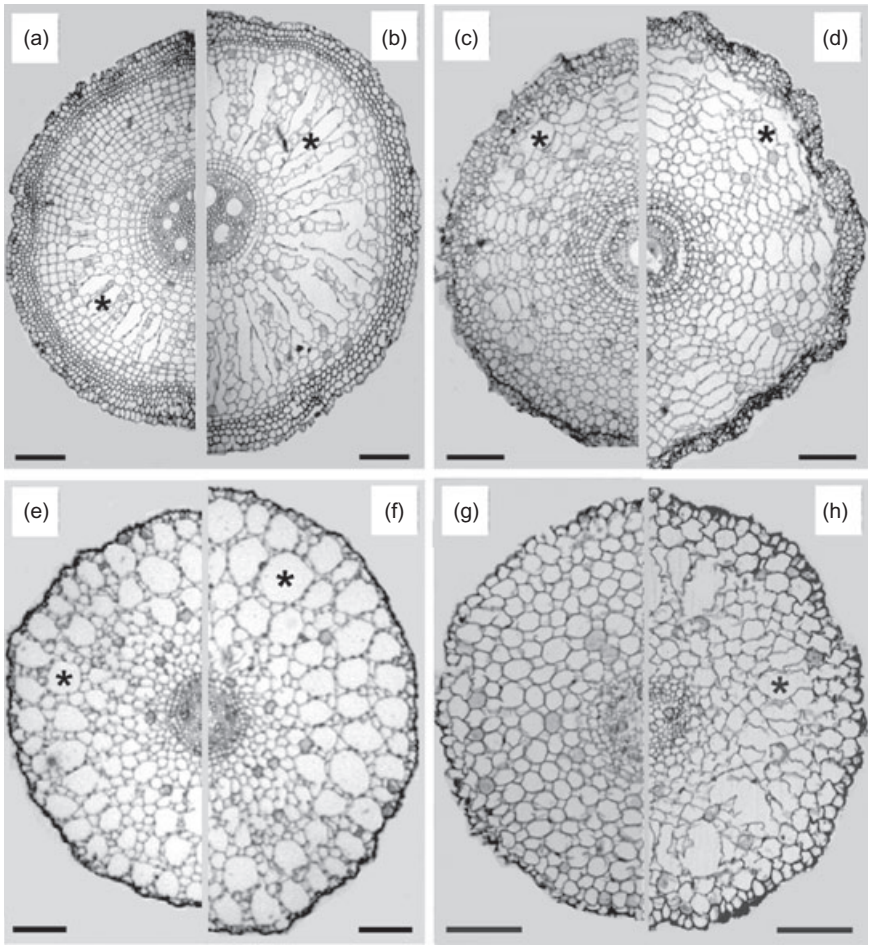

Figure 3. Cross sections of roots of (a & b) *Paspalidium geminatum*, (c & d) *Cyperus eragrostis*, (e & f) *Rumex crispus* and (g & h) *Plantago lanceolata* plants grown for 30 d under control (a, c, e & g) and flooded (b, d, f & h) conditions. Cross sections were made at 3 cm above the root apex. Asterisks indicate aerenchyma lacunae. Bars = 150 μm.

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Table 2. Ratio between area of epidermis plus outer cortex tissue and the perimeter of the inner cortex

Species	Ratio $\frac{\text{area epidermis + outer cortex (mm}^2\text{)}}{\text{Inner cortex perimeter (mm)}}$	
	Control	Flooded
Paspalidium geminatum	0.118 ± 0.011	0.126 ± 0.017
Cyperus eragrostis	0.101 ± 0.005	0.093 ± 0.008
Rumex crispus	0.012 ± 0.002	0.011 ± 0.004
Plantago lanceolata	0.021 ± 0.004	0.028 ± 0.003

Values are means ± SE ($n = 5$).

root diameter or the occurrence of lysogenic processes (Fig. 4). Contrarily, root structural types that presented only the uniseriate epidermal layer (*Rumex* and *Apium* types) to cope compression were progressively weakened as root porosity increased. In *R. crispus*, the weakness of the root system was completely associated with the increment in root diameter under both control and flooded conditions (Fig. 4 & Table 3). Importantly, in the control roots of *P. lanceolata*, the mechanical strength was not affected by the increase of root diameter (Fig. 4), but root porosity was low (without lacunae in the root cortex) and only consisted of small intercellular air spaces related to the cubic configuration of the cells (Fig. 3g). The flooded roots of *P. lanceolata* were the only case in which root diameter and cell lysis were additive in increasing porosity, and therefore promote weakness of the root system (Fig. 4).

In *P. geminatum*, *C. eragrostis* and *P. lanceolata*, the formation of additional aerenchyma by lysis was made evident by the increase in the slope of the relationships between porosity and diameter of flooded roots in comparison to control roots (Fig. 4, Table 3). In these species, the number and maximum size of lacunae in the cortex were considerably higher in flooded than in control roots (Fig. 3 & Table 4). As expected, in *R. crispus* (species with expansigenous aerenchyma) the increase in the proportion of cortex as a function of root diameter was closely related to the increment in root porosity under both control and flooded conditions (contrast Fig. 3e & f). This

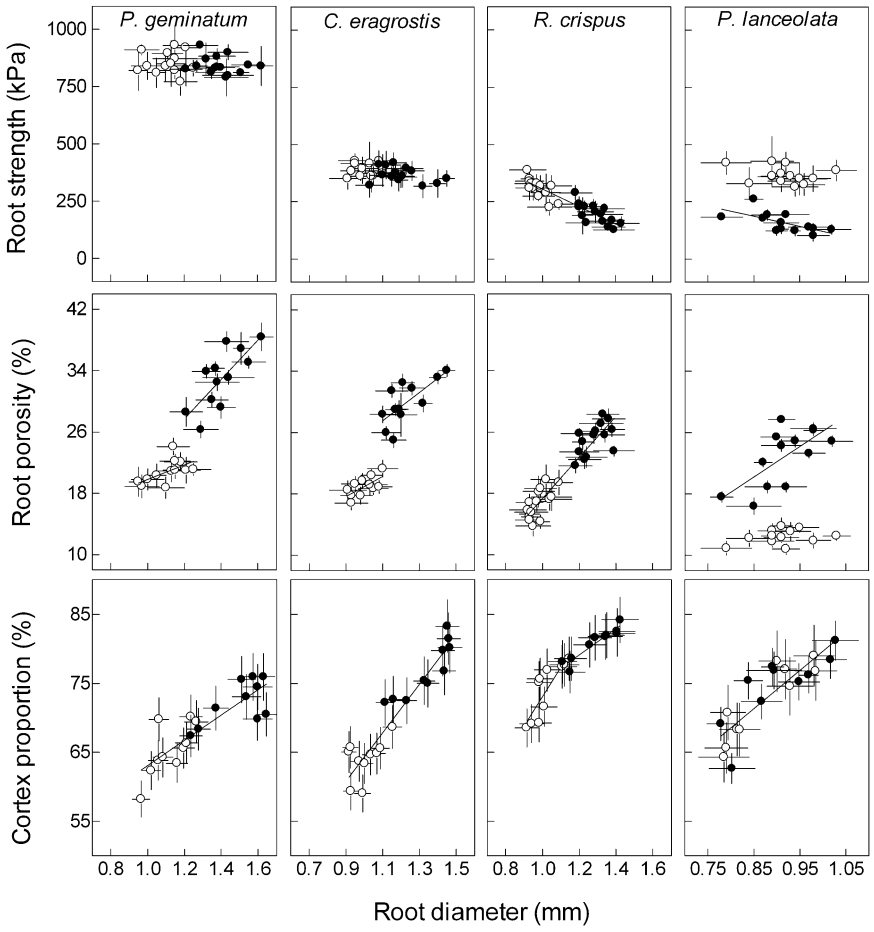

Figure 4. Root strength, root porosity and cortex proportion in relation to root diameter in *Paspalidium geminatum*, *Cyperus eragrostis*, *Rumex crispus* and *Plantago lanceolata* plants grown under control (○) and flooded (●) conditions. Solid lines represent significant regressions ($n = 10$–14, $P < 0.05$; see Table 3). When differences between parameters of control and flooded roots were not detected, data were pooled to obtain a single linear regression. Values represent averages ± SE of four to five roots per plant.

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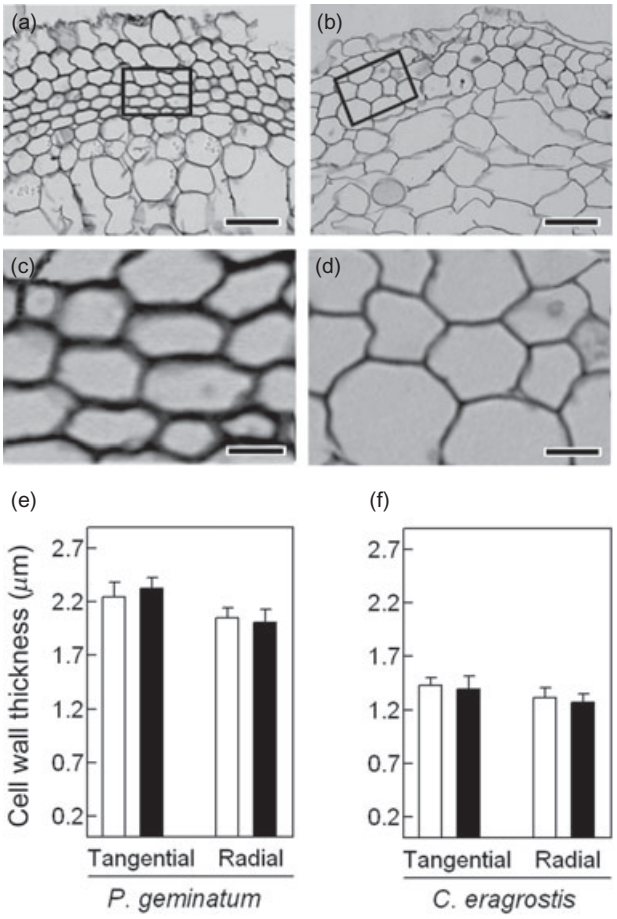

Figure 5. Epidermis, outer and inner cortex of roots of (a) *Paspalidium geminatum* and (b) *Cyperus eragrostis*. Bars = 60 μm. Magnified sections of the sub-epidermal zone enclosed in (a) and (b) showing differences of cell wall thickness in (c) *P. geminatum* and (d) *C. eragrostis*. Bars = 15 μm. Tangential and radial cell wall thickness in the outer layers of the cortex of (e) *P. geminatum* and (f) *C. eragrostis* roots grown for 30 d under control (□) and flooded (■) conditions. Values are means ± SE ($n = 5$).

was indicated by the single linear relation between control and flooded treatments (Fig. 4 & Table 3). Flooded roots of this species showed a higher number of lacunae in the root cortex, but their maximum size was only slightly bigger in relation to control roots (Fig. 3 & Table 4). In this case, the newest aerenchyma lacunae were generated by the expansion of pre-aerenchymatous spaces located in the inner section of the root cortex in contrast to the rest of species, in which the number of aerenchyma lacunae was achieved by cellular lysogenic processes (Fig. 3). Interestingly, this species attained the highest value of cortex proportion under flooded conditions, but showed a relatively lower slope for flooded roots in comparison to control ones (Fig. 4 & Table 3). This result suggests the presence of a morphogenetic plateau for this variable (Fig. 4), and that further increases of cortex proportion could not be expected.

DISCUSSION

Root anatomical traits underlying the differential effects of porosity on root strength

The results showed that any increase of root porosity, even as a result of root diameter and/or lysogenic processes, weakened root structure in species that lacked a multiseriate ring of densely packed cells in the outer layers of the cortex. Both structural types presenting a uniseriate epidermal layer (*Rumex* and *Apium*) were progressively weakened throughout the incremental range of root porosity. In contrast, structural types possessing a multiseriate ring protecting the cortex (graminaceous and cyperaceous) did not lose strength, having even duplicated their root porosity under flooding conditions. In these last species mentioned, the number of cell layers and cell wall thickness in epidermal and sub-epidermal tissues appeared to determine the strength of the roots.

In both species with strong roots, *P. geminatum* and *C. eragrostis*, the multiseriate ring was internally reinforced by rows of parenchymatous cells in the inner cortex, which were perpendicularly aligned in the direction of the mechanical forces. In addition, *C. eragrostis* presented rows tangentially connected by remnant cell walls. Such particular properties enable the formation of physically stable structures that add strength to the roots (Niklas 1999; Striker *et al*. 2006). However, a significant difference in mechanical strength was registered for *P. geminatum*, which required double the pressure to collapse in comparison with *C. eragrostis*. This difference appears to be related to the mechanical support that the presence of sclerenchyma tissue brings to organ structures (Niklas 1992; Li *et al*. 2003). In accordance, *P. geminatum* possessed three to four layers of sclerenchymatous cells in the outer layers of the cortex, with thicker cell walls (observed in tangential and radial axes) as compared with *C. eragrostis*. Interestingly, root traits preventing ROL – presence of thicker cell walls and suberin deposits in epidermal and sub-epidermal layers (Clark & Harris 1981; Colmer *et al*. 1998; Armstrong *et al*. 2000) – appeared to be a determinant in the higher mechanical strength of these root structural types. The possible double function of such root traits merits further experimental investigation.

In *R. crispus* and *P. lanceolata*, the decreased strength of the root structure was closely related to the increment in porosity of the root cortex. In these species, the formation of aerenchyma tissue facilitates oxygen transport to submerged tissues (Armstrong 1979; Laan *et al*. 1989; Colmer 2003), but dramatically altered the internal structure of the roots so that they collapse at progressively lower pressures. For both species, this mechanical behaviour appears to lie in the decreasing proportion of cell walls per unit of root volume (Niklas 1992). In our study, this was clearly related to the enlargement and generation of aerenchyma lacunae in the root cortex. Accordingly, Kokubo *et al*. (1989) stated that the proportion of cell walls (i.e. cellulose content) was a key parameter in defining the mechanical strength of barley stems. In addition, the extent in which neighbouring

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Table 3. Linear regression analysis of the relationship between root strength, root porosity, cortex proportion and root diameter

Variable	Species	Treatment	Slope	Intercept	r^2
Root strength	*Rumex crispus*	Control and flooded	–440 (30)	680 (40)	0.88
	Plantago lanceolata	Flooded	–380 (150)	560 (140)	0.42
Root porosity	*Paspalidium geminatum*	Control	9.5 (3.1)	10.2 (4.5)	0.35
		Flooded	25.7 (5.6)	–3.1 (9.5)	0.59
	Cyperus eragrostis	Control	11.6 (3.5)	–1.6 (6.7)	0.49
		Flooded	20.9 (6.1)	11.1 (6.5)	0.52
	R. crispus	Control and flooded	23.4 (1.8)	–9.3 (2.1)	0.88
	P. lanceolata	Flooded	21.3 (5.4)	–13.6 (11.7)	0.47
Cortex proportion	*P. geminatum*	Control and flooded	18.4 (2.7)	44.7 (3.6)	0.73
	C. eragrostis	Control and flooded	35.1 (2.8)	29.3 (3.4)	0.89
	R. crispus	Control	33.4 (8.7)	30.6 (10.0)	0.70
		Flooded	19.0 (2.4)	56.3 (3.1)	0.88
	P. lanceolata	Control and flooded	35.2 (5.6)	24.1 (7.1)	0.72

Equations with significant parameters ($P < 0.05$) are shown. When differences between slopes were not detected, data were pooled to obtain a single linear regression. Values in brackets are SE ($n = 10$–14).

cells of porous tissues hydrostatically reinforce one another is known to be a function of the cell-to-cell contact area (Niklas 1992). For *P. lanceolata*, it is possible that the close contact among cells where the aerenchyma tissue is not developed could ameliorate the lack of mechanical tissue (e.g. sclerenchyma) in the cortex. Under flooding conditions, however, cell lysis greatly increased root porosity, producing a minimum contact among cortical cells. In this situation, the generation of irregular aerenchyma lacunae (*Apium*) seems to enhance the deformation of the root structure in the presence of compressive forces.

As expected, control roots of the studied species appeared to be strong enough (350–450 kPa) to tolerate pressure values occurring under field conditions (120–300 kPa; Richards & Greacen 1986; Scholefield & Hall 1986; Kirby & Bengough 2002). Moreover, in *P. geminatum* and *C. eragrostis*, the maintenance of root integrity even with an increased extent of porosity indicates that they would also tolerate soil compression under flooded conditions. This last result is in line with the positive relation between mechanical root strength and tolerance to cattle trampling in flooded soil in the grass *P. dilatatum* (Striker *et al*. 2006). In contrast, in *R. crispus* and *P. lanceolata*, the progressive loss of root mechanical strength under increasing porosity (120–150 kPa to collapse) is in accordance with previous experiments reporting a poor performance of these root structural types when they were simultaneously subjected to flooding and soil mechanical stress (Engelaar *et al*. 1993b; Engelaar & Blom 1995; Striker *et al*. 2006). Particularly in *R. crispus*, this result is in accordance with the full collapse of the roots of *Rumex palustris* (with similar root type) reported by Engelaar *et al*. (1993b) when hypoxia and soil compaction treatments were combined. In this regard, our study helps to explain why both *R. crispus* and *R. palustris* are more successful and frequent under extremely wet conditions but not on compacted flooded soils (Blom *et al*. 1994; Engelaar & Blom 1995). For *P. lanceolata*, our results are in accordance with those obtained for *L. glaber* (*Apium* root type), which did not tolerate mechanical stress by simulated cattle trampling in flooded soil (Striker *et al*. 2006). In this respect, we did not find secondary growth in any root cross sections observed of *R. crispus* and *P. lanceolata* (3 cm behind the apex) for either control or flooded plants (data not shown). Although the literature reports the suppression of secondary growth as a consequence of flooding (Justin & Armstrong 1987; Jackson & Armstrong 1999; Grimoldi *et al*. 2005), it is probable that if such growth occurs before soil drying, the mechanical strength and survival of dicotyledonous roots could be enhanced. The role of secondary growth in conferring

Table 4. Aerenchyma lacunae and mean maximum area per lacunae of adventitious roots grown for 30 d under control and flooded conditions

	Aerenchyma lacunae			
	Number		Mean max area ($\times 10^2$ μm^2)	
Species	Control	Flooded	Control	Flooded
Paspalidium geminatum	23.0 ± 1.8	33.8 ± 1.4**	43.3 ± 3.4	81.4 ± 3.8**
Cyperus eragrostis	61.0 ± 5.5	102.0 ± 8.5**	22.5 ± 2.8	41.1 ± 3.6**
Rumex crispus	88.0 ± 5.1	111.5 ± 8.7*	70.2 ± 6.7	95.3 ± 9.1*
Plantago lanceolata	3.5 ± 0.9	23.8 ± 1.5**	39.4 ± 7.5	136.6 ± 7.9**

Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$) between treatments within species based on Student's *t*-test. Values are means ± SE ($n = 5$).

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mechanical resistance to *Rumex* and *Apium* root structural types merits further study.

Relationship between aerenchyma formation and mechanical strength at different root diameters

Previous works have studied in detail the processes of aerenchyma formation and plant internal aeration (Armstrong 1979; Justin & Armstrong 1987; Jackson & Armstrong 1999; Colmer 2003; Seago *et al*. 2005 and references therein). However, the scaling among anatomical traits, mechanical properties and root size was never explored. Interestingly, we found that the proportion of cortex fully correlated with root diameter under both control and flooded conditions. The increase in the proportion of cortex as a function of root diameter registered in all species scaled positively with the increment of root porosity as aerenchyma and intercellular airspaces are located in the cortical tissue.

In spite of the general pattern, the importance of diameter or cell lysis in increasing porosity differed among root structural types. In the control plants of *P. geminatum* and *C. eragrostis*, the slight but significant increase in root porosity, together with the diameter, was in accordance with the increase in the proportion of root cortex in which the aerial spaces are located. In both species, a much higher slope between the porosity and the diameter of flooded roots indicates that cell lysis largely adds porosity to the root system. Moreover, the higher number and size of aerenchyma lacunae registered in flooded plants, both common responses to anoxic stress (Justin & Armstrong 1987), support this idea. Increased root diameter and lysogenic processes in response to flooding have been reported in graminaceous and cyperaceous species (Visser *et al*. 2000; Grimoldi *et al*. 2005), and have been interpreted as a functional root response for enhancing oxygen diffusion (Colmer & Greenway 2005). In our study, the increment of root porosity produced by cell lysis and by changes in root diameter did not affect the root strength of these species. Therefore, it could be expected that the maintenance of root integrity allowed those species to continue root oxygenation when subjected to external mechanical pressures.

In *R. crispus*, the increase of porosity was achieved through the progressive expansion of intercellular air spaces at increasing root diameter (and cortex proportion) by oblique cell divisions in radial rows of the cortex (Justin & Armstrong 1987). Accordingly, the increase in root diameter by flooding (or hypoxia) is a common response in *Rumex* species (Engelaar *et al*. 1993b; Visser *et al*. 2000). In this species, the new aerenchyma lacunae were related to the growth of pre-aerenchymatous spaces in the inner section of the cortex as previously reported (Laan *et al*. 1989; Seago *et al*. 2005). Therefore, for this root structural type, the progressive weakness of the root was mainly related to the increment of root diameter. In *P. lanceolata*, no correlation between diameter and root strength was found under control conditions in which aerenchyma lacunae were not developed. However, the flooded roots of *P. lanceolata* were the only case in which root diameter and cell lysis seemed to be additive in weakening the root structure. Under flooded conditions, the formation of aerenchyma lacunae by cell lysis determined that the incremental proportion of cortex (correlated with root diameter) also appeared as a significant weakening factor.

CONCLUSIONS

This study demonstrates a strong trade-off between aerenchyma formation and mechanical strength in root structural types that lacked a multiseriate ring of cells for mechanical protection in the outer layers of the cortex. The effect was significant when the increment of root porosity was solely because of increased root diameter, or when cell lysis also added porosity to the roots. The results also suggest that this ring of cells plays a significant role in maintaining the mechanical strength of roots when flooding induces the generation of additional aerenchyma tissue in the cortex. Further experiments are now needed to evaluate whether the physical barriers that help strengthen roots share function with those preventing oxygen loss in the root system, and to test if secondary growth brings mechanical protection to dicotyledonous roots.

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