

Root lodging tolerance in *Helianthus annuus* (L.): associations with morphological and mechanical attributes of roots

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Received: 10 October 2013 / Accepted: 11 April 2014 / Published online: 21 April 2014
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Abstract

Background and aims The objectives of this study were to quantify the morphological and mechanical properties of the root-plate within two sunflower hybrids of contrasting susceptibility to root lodging; and to evaluate the effects of crop population density on these properties at two different development stages.

Methods Two hybrids (CF29: tolerant, Zenit: sensitive) were grown at three densities: 5.6, 10 and 16 plants m⁻². At R2 (early reproductive) and R6 (end anthesis) development stages, plants were artificially lodged and stem biomass, total root biomass in the whole root-plate and in the 0–5 and >5 cm layers of the plate, root number (three diametrical categories: 0–1, 1.1–2, >2 mm), total root length, and root axial breakage force were assessed.

Results CF29 root mass was twice that of Zenit with differences mainly in the top 5 cm of soil. This higher root-plate biomass of CF29 was associated with a greater root number and root length compared to Zenit within all root diameter categories. Roots of CF29 exhibited higher axial tension failure thresholds than those of Zenit, and these thresholds increased more sharply with root diameter in CF29 than in Zenit.

Conclusions The better anchorage and tolerance to lodging of CF29 with respect to Zenit arose from additive actions of traits at both whole root-plate and individual root levels. These included total root-plate root

length, root number, root biomass and root axial breakage force.

Keywords Crop population density · Ontogenetic stages · Root-plate biomass · Root-plate diameter · Root length · Root number · Root axial breakage force

Introduction

The support structures of terrestrial plants must resist the combined effect of gravity and wind loads (Niklas 1992). In a wide range of crops plant anchorage failure leading to root lodging can occur, resulting in serious adverse effects on yield (Baker et al. 1998) and grain quality (Kelbert et al. 2004). In Argentina around 10 % of the sunflower crop area suffers lodging every year with yearly losses have been estimated at US\$40 million (Bragachini et al. 2001).

Root lodging occurs when the stems remain intact, but the soil and roots supporting the plant fail and movement of the root-plate (the hemisphere of roots and soil formed when lodging occurs) ensues (Pinthus 1973). Root lodging is mainly associated with rain (which diminishes soil shear strength) (Baker et al. 1998) combined with wind that exerts a force acting on the upper sections of the plant resulting in a bending moment sufficient to cause breakage of windward roots, thus allowing the root-plate to rotate (Berry et al. 2003a, b). The mechanical loading roots experience is determined by: 1) the general morphology of the root system, which dictates how the stresses are spread along the roots of—the root plate (Ennos 1993; Crook and Ennos 1996; Stokes and Mattheck 1996); 2) the morphology and the

Responsible Editor: Alain Pierret.

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absolute size of the aerial portion of the plant, which determine how mechanical forces are transmitted to the root system (Ennos 1993); and 3) soil and weather conditions, which influence the ability of the roots to anchor plants to their substrates in the face of forces imposed on leaves and stems (Niklas 1992). In our paper we focus on the first of these three points to the exclusion of the other two, in an attempt to disentangle the relationships between root-plate diameter and the root morphological and mechanical properties. Our approach differed from that of Ennos (1993) in that we were not concerned about the presence/absence of the tap root (both genotypes were tap-rooted), but on the properties of the other roots of the root-plate. From that of Crook and Ennos (1996) our study differed in that they studied rooting patterns of trees (rather than herbaceous plants) and their relationship with anchorage behavior. Finally, we differed from that of Stokes and Mattheck (1996) in that they studied the shape of root system of different tree species in relation with the mechanical traits in uprooting situations, with special emphasis on soil reinforcement rather than lodging tolerance. In relation to the general morphology of the root system, the likelihood of root lodging depends on many factors relating to roots (e.g., root biomass, stem-root biomass allocation pattern; root distribution with depth; root number, length and distribution across different diameter classes; Burylo et al. 2009, 2012). Although there are some papers about root anchorage in different species (see Burylo et al. 2009; De Baets et al. 2008), all of them have been focused mainly in soil erosion processes, while our approach was focused on plant lodging.

It is known that a higher biomass allocation to roots can improve anchorage strength (Cleugh et al. 1998); hence the need to examine the effects of variations in stem-root biomass ratio on this strength. However, root-plate root biomass is not the only feature contributing to lodging tolerance. At the whole root system level, architectural design is an important trait related with anchorage function of plants. Several anchorage systems are possible in order to optimize the mechanical strength of roots, and this is dependent on plant size (Crook and Ennos 1998; Bailey et al. 2002). Some examples are tap-root systems in some herbaceous species such as *Helianthus annuus* (Ennos et al. 1993), and trees such as *Picea sitchensis* that produce thick woody lateral roots (Couts 1983, 1986; Crook and Ennos 1996). Properties of the anchorage system in annual crops have been related to root lodging tolerance, such as root

number, root biomass and root length density (Ennos and Fitter 1992; Hébert et al. 2001; Burylo et al. 2012). We addressed this dimension of the issue by studying these aspects of root morphology, and examining the associations between root lodging tolerance and total root plate root length and root diametrical categories in two sunflower hybrids known to differ in susceptibility to lodging. To our knowledge, ours is the first study involving a crop species in which these last two associations have been explored using genotypes of known contrasting susceptibility to root-lodging.

At the individual root level, root axial breakage force and its relationship with root diameter are attributes strongly associated with lodging tolerance. In trees (both conifer and broadleaf species, Bischetti et al. 2005; Genet et al. 2005), and in the cereal model plant *Hordeum vulgare* (Loades et al. 2010, 2013) the main properties of individual roots related to anchorage strength are root tensile strengths and diameter. In other species of agronomic interest such as rice the relationship between these properties have not yet been established (Oladokun and Ennos 2006), and for sunflower there are no records yet of this kind of approach. Given that root lodging starts with the breakage of windward roots, axial breakage force of these roots that form part of the root-plate in sunflower plants could be important in determining likelihood of root-plate rotation. Therefore our approach aimed at establishing the relationship between root axial breakage force and diameter in relation to lodging in sunflower.

Crop population density (CPD) affects several plant characteristics associated with lodging. With the magnitude of these effects also dependent on genotype (Berry et al. 2007). In maize, increasing crop population density, associated with reductions in light levels within the canopy and changes in light quality (low Red/Far Red ratio), affect morphology and biomass allocations patterns, increasing susceptibility to lodging (Guingo and Hébert 1997; Hébert et al. 2001; Pellerin and Demotes-Mainard 1992). In wheat high CPDs decreased the number of roots (Berry et al. 2000; Easson et al. 1995), while an increase in CPD in sunflower reduced root-plate diameter (Sposaro et al. 2008). However, the effects of variations in CPD on root-plate root properties such as root morphology and axial strength failure thresholds have not yet been explored in any crop species.

In cereal crops, the greatest likelihood of lodging coincides with heading and early grain development

stages (Easson et al. 1993; Pinthus 1973), while in sunflower Sposaro et al. (2008) showed that root failure moment and root-plate diameter increased with the crop development between the R2 (early reproductive, distance from floral bud to uppermost leaf between 0.5 and 2.0 cm) and R6 (end anthesis) stages (Schneiter and Miller 1981). They observed little change later in the season, and that genotype influenced these changes such that the greatest differences between genotypes were exhibited at R6. To date, there have been no studies on the morphological and mechanical properties of the roots that form the root-plate in sunflower plants and their variation with genotype, crop population density and crop ontogenetic stage.

The objectives of the present study were, first, to quantify the differences in morphology and mechanical properties of root-plate roots at the whole root-plate and individual root levels in two sunflower hybrids of contrasting tolerance to root lodging; and second, to evaluate the effects on these variables of different levels of CPD at the R2 and R6 development stages. We hypothesized that: i) The differences in root lodging tolerance between the hybrids are associated with the morphological and mechanical properties of root-plate roots at both the whole root-plate and individual root levels; and ii) Increases in CPD have a negative effect on root-plate root attributes associated with root lodging tolerance across genotypes and crop developmental stages.

Materials and methods

Experimental design and crop growth conditions

A first experiment was carried out using two genotypes of sunflower of contrasting susceptibility to root lodging (CF29, Advanta Seeds, Argentina; Zenit, Sursem, Argentina). Sposaro et al. (2008) showed that the root failure moment of CF29, grown at a crop population density of 5.6 pl m⁻², was 1.6 and 3 times greater than that of Zenit at the R2 and R6 development stages at which measurements were made, respectively. Crops were sown 08/12/07 on a silty clay loam soil (Typic Argiudoll) at the Faculty of Agronomy, University of Buenos Aires (FAUBA) (34°35'S, 58°29'W). A randomized complete block design with three replicates was used with “genotype” (two levels: CF29 and Zenit) and “Crop Population Density” (CPD, three levels: 5.6, 10 and 16 plants m⁻²) as principal factors.

Crops were over-sown by hand in rows spaced at 0.7 m and thinned at the V4 stage (four true leaves of at least 4 cm in length; Schneiter and Miller 1981) to the stipulated CPDs. Plots were six rows (including two border rows) wide by six meters long. Crops were protected against weeds, insects and diseases as needed and were irrigated and fertilized to prevent water and nutritional stresses. A second experiment, sown on 10/12/08, was conducted using the same conditions in order to obtain (in addition to measurements of root-plate diameter and root failure moment) information on partitioning of total plant biomass between root-plate and stem for both genotypes. This second experiment was sown using only two CPDs (5.6 and 10 plants m⁻²) and harvests were made at the R2 and R6 stages. During both experimental periods (2007/08 and 2008/09 summer seasons), environmental conditions were similar. Mean daily temperature was 23.7±0.2 and 24.9±0.1 °C; and mean daily radiation was 25.3±1.2 and 24.6±1.7 MJ m⁻² d⁻¹ in Exps. 1 and 2, respectively. Seasonal cumulative rainfall was also similar between years (372 and 361 mm, respectively), although this variable was not relevant because crops were irrigated as needed in both years.

Artificial root lodging

Root failure moment measurements were performed at R2 and R6 stages in both experiments. The soil of one subplot per experimental plot (1.6 by 0.7 m centred on the row and enclosing six plants) was pre-wetted 48 h before measurements were made. To do this, steel plates with slotted ends were hammered into the soil to a depth of 20 cm, leaving a further 10 cm projecting above soil level, forming a rectangular soil monolith. Water was repeatedly added to the soil surface to ensure that soil was fully wetted to a depth of 30 cm. Three contiguous plants per block (18 plants per ontogenetic stage) were artificially lodged using the instrument described by Sposaro et al. (2008). These plants were in perfect competition with neighboring plants within the row and with those in the two flanking rows and were separated by at least one plant from the shorter side of the sub-plot. After removal of the leaves and the capitulum, the instrument push-bar was set at 60 % of the plant height (h) in contact with the stem. A force was applied to contact point with the stem, where it was attached by means of steel cable to a windlass and pulley system linked to a balance (Model HEC, Balanzas

Electronicas Torres). The stem was incrementally displaced from its vertical position until root lodging occurred. At the end of each incremental displacement, the force at the balance, the angle between the cable and the push-bar, and the angle of displacement of the stem from the vertical were registered. Values recorded at the balance were transformed using standard decomposition of forces procedures (Sposaro et al. 2008), to estimate the force (Nm) needed to induce root lodging.

Root-plate root morphology

Once lodging had taken place, the roots included in the root-plate were harvested and washed with water and separated into two layers: from the shoot base to 5 cm of the tap root (layer 1), and >5 cm to the lower limit of the root-plate (layer 2). The criterion for the use of these strata was based on a) around 70 % of the biomass of secondary roots is located in the first centimetres of soil (Ennos et al. 1993, and our Fig. 2), and b) our observations of root plates which indicated that root length density dropped away sharply at depths greater than 5 cm. The roots from each layer were separated and classified in three categories according to their diameter: fine, 0.1–1.0 mm; medium, 1.1–2.0 mm; thick, >2.0 mm. Root diameters were measured using digital thickness gauge (Köln, Germany) in three different positions along its length to obtain an average diameter.

Biomass, length and number of root-plate roots

Lateral roots included in the root-plate obtained after artificial root lodging were excised from the tap-root, and in each of the two layers of the root-plate, the length and number of lateral roots in each diameter category were determined. Estimation of root-plate soil volume proved complex in some instances due to irregular root-plate shape. Hence, we report root length values rather than root length density ones because the former was a more powerful discriminant variable than the latter and was less subject to error. All subsamples were weighed after being oven-dried at 80 °C for 48 h to determine their biomass (Gregory 2006). The values of tap root length and biomass were not included in any analyses.

Root axial breakage force

Samples of individual roots of each of the three diameter categories from each of the two root-plate layers

were separated and subsamples that showed no visible damage associated with the lodging or harvesting were used to measure the axial force necessary to break them. The selection of undamaged roots was done by eye, selecting those roots connected to the tap-root which retained their apices. Values for any roots which broke, during the test runs, close to the clamps which held the root samples were discarded. To measure the root-plate root axial breakage force a technique modified from that described by Striker et al. (2006) was used. Briefly the technique consists in applying an increasing horizontal force to a root section until it broke and registering the peak tensile force required to produce breakage. The device allowed the two extremities of a root section to be held with clamps specifically designed to avoid root damage and rupture at the clamping points. Each clamp consisted of a 19-mm wide binder clip, the internal faces of which were lined with soft 2-mm thick wooden patches. These lined clips substantially reduced the levels of root breakage close to the clamp with respect to results obtained using unlined clips. A piston contained in a minicylinder and connected to a pneumatic circuit allowed tension to be applied to the clamps. 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 breaking (transducer error: ± 0.0005 bar). The root axial tension breaking threshold (N) was calculated as: $F = \text{pressure in the cylinder (kPa)} \times \text{internal piston area (cm}^2\text{)}$ (Ennos 1990).

Stem-root plate biomass ratio

In the second experiment whole plants were harvested after lodging and separated into stem and root-plate roots (including the tap-root) after removing the soil of the root plate by washing. All samples were weighed after being oven-dried at 80 °C for 48 h (Gregory 2006) and the ratio between stem and root-plate biomass was calculated.

Statistical analyses

The stem-root plate biomass ratio, root biomass, root length and root number data were analyzed using three-way ANOVAs with ontogenetic stages (OS), genotypes (G) and crop population density (CPD) as main factors. An a posteriori Tukey test was used to identify significant ($P \leq 0.05$) differences between treatments.

Normality and the homogeneity of variable variances were previously verified. Variables involving proportions were arcsine \sqrt{x} transformed before analyses. Statistical analyses were performed using the InfoStat/Professional V2006 P.2 (Di Rienzo et al. 2010) package. Variable values are shown as untransformed means \pm one standard error even when significance of treatment effects were determined using transformed values. Pearson correlations were used to evaluate the relationship between root axial breakage force and root diameter (Steel and Torrie 1988). The differences between genotypes were evaluated by applying the slope test (F-test) to the fitted equations.

Results

No significant ($P > 0.05$) differences were found between the two experiments in the responses of root failure moment (R_{fm}) and root-plate diameter to genotype (G), crop population density (CPD) and ontogenetic stage (OS) (data not shown). Consequently, and in what follows, we illustrate these responses with data from Exp. 1, and show data from Exp. 2 pertaining to stem-root plate biomass ratio, a variable which was only measured in Exp.2. There were no significant two- or three-way principal factor (G, CPD, OS) interactions for most of the measured variables (Table 1 only shows those interaction that were significant in at least some principal factor combinations). There were effects of genotype (G), crop population density (CPD) and ontogenetic stage (OS) on all variables described below ($P \leq 0.05$; Table 1) except on root axial breakage force where CPD and OS did not have any effect.

Root-plate failure moment

The main differences in root failure moment (R_{fm}) were those between genotypes and between ontogenetic stages within each genotype (G: $P < 0.0001$; OS: $P < 0.001$), with both genotypes showing a lower R_{fm} with increasing CPD at both development stages, although CF29 always had the greater R_{fm} (Table 2). Across CPDs (d1: 5.6, d2: 10 and d3: 16 plants m^{-2}), differences between d1 and d2=d3 tended to become clearer in R6 with respect to R2. At R2, CF29 had a significantly ($P < 0.05$) greater (*ca.* three times higher) root failure moment than Zenit at the lowest CPD, but these differences were not significant at higher CPDs.

At R6 and for all CPDs there were significant differences in favour of CF29 (Table 2).

Root-plate diameter

Root-plate diameter showed a significant ($P < 0.05$) OS*CPD interaction (Table 1) which was linked to the contrasting responses to CPD of Zenit in R2 (no significant response) and R6 (significant response, d1 different to d2=d3). In addition, and more importantly, results reveal significant responses to all three principal factors (Tables 1 and 2). CF 29 showed much greater root-plate diameters than Zenit, especially at R2 (on average the CF29/Zenit differences were 23 % and 9 % at R2 and R6, respectively; Table 2). The effect of increasing crop population density on root-plate diameter was significant ($P < 0.001$) with values for this variable decreasing as CPD increased in both genotypes (compare d1 vs. d2=d3; Table 2). As ontogeny progressed from R2 to R6, root-plate diameter increased in d1 and d2 in both genotypes (Table 2).

Total root-plate root biomass

The total biomass of root-plate roots showed a significant ($P < 0.05$) OS * G interaction (Table 1). This interaction arose from the lack of an OS effect in Zenit at the d2 and d3 densities, while root-plate biomass increased with OS at all densities in CF29 (Table 2). Total root-plate root biomass differed significantly ($P \leq 0.05$) between genotypes and OS's, but not among CPD's (Table 1). CF29 had close to twice the root-plate root biomass of Zenit across the two OS and the three crop population densities tested (Table 2). At the R2 OS the crop population density effect was not significant for either of the genotypes, although root-plate biomass tended to fall with increasing CPD ($P = 0.35$, Table 2). This tendency became more evident at the R6 OS, with significant differences ($P < 0.05$) between CPDs d1 and d2=d3 in both genotypes (Table 2).

When root-plate root biomass was analyzed separately for the two layers of the root-plate, there was an effect of genotype (G: $P < 0.05$, Table 3). At the R2 stage and in d1, CF29 had 67 % of its root biomass in the first 5 cm, while Zenit had similar proportions of root biomass in both layers (48 and 52 % for Layers 1 and 2, respectively). Increased crop population density had no effect on the proportional distribution of root biomass in the root-plate in either genotype at this stage ($P > 0.05$).

Table 1 Summary ANOVA table for effects of principal factors: Ontogenetic Stage (OS), Genotype (G) and Crop Population Density (CPD) on root-plate properties. Data are *F* values test results. Two and three-way interactions between principal factors were

non-significant except for those shown in the OS*CPD and OS*G columns. Data from Exp. 1, with the exception of the stem/root-plate root biomass ratio, which was measured in Exp. 2

Root-plate properties	Principal factors			Interactions	
	Ontogenetic stage	Genotype	CPD	OS*G	OS*CPD
Root failure moment	54.80**	32.39**	5.10**	11.69ns	0.05ns
Root-plate diameter	8.64*	32.67**	21.28**	2.13ns	6.26*
Total root-plate biomass	16.53**	40.16**	0.62ns	4.34*	2.23ns
Root-plate biomass layer 1	15.82**	31.84**	1.35ns	1.44ns	1.79ns
Root-plate biomass layer 2	3.84*	0.98*	4.81*	1.18ns	3.74*
Root number	0–1 mm	5.68*	9.78**	1.09ns	1.35ns
	1.1–2 mm	6.02*	5.13*	1.72ns	2.63ns
	>2 mm	4.95*	7.06*	1.18ns	2.31ns
Total root length	0–1 mm	6.65**	28.02**	0.89ns	0.12ns
	1.1–2 mm	7.29**	21.17**	1.31ns	0.09ns
	>2 mm	0.47ns	5.37*	0.23ns	0.91ns
Stem: root-plate biomass ratio	16.97**	20.52**	5.71*	0.41ns	0.01ns
Root axial breakage force	0.99ns	50.07**	0.89ns	0.63ns	0.19ns

*Indicates significance at $P \leq 0.05$

**Indicates significance at $P \leq 0.01$

ns not significant $P > 0.05$

Unlike what was observed in the R2 stage, at the R6 stage both genotypes had the highest ($P < 0.05$) root biomass concentrated in the first layer (0–5 cm): 78 % to 71 % for CF29 and Zenit respectively (Table 2). In

this layer, root biomass of CF29 was 74.4 % higher ($P < 0.05$) than in Zenit. In the second layer (>5 cm) the root biomass was also higher ($P < 0.001$ Table 2) for CF29. There was a negative effect ($P < 0.01$) of crop

Table 2 Root-plate diameter and failure moment; stem/root-plate root biomass ratio and total root biomass for the genotypes CF29 and Zenit for three CPDs (d1: 5.6; d2: 10 and d3: 16 plants m^{-2}) at

the R2 and R6 development stages (floral bud and end of anthesis, respectively). Data from Exp. 1, with the exception of the stem/root-plate root biomass ratio, which was measured in Exp. 2

	Development stage	CF29 Crop population density			Zenit Crop population density		
		d1	d2	d3	d1	d2	d3
Root failure moment (Nm)	R2	2.9±0.28 <i>Aax</i>	1.2±0.24 <i>Abx</i>	0.8±0.16 <i>Abx</i>	1.0±0.12 <i>Bax</i>	0.7±0.11 <i>Aax</i>	0.5±0.03 <i>Aax</i>
	R6	8.01±2.07 <i>Aay</i>	5.2±0.86 <i>Aby</i>	5.0±1.01 <i>Aby</i>	3.0±0.80 <i>Bay</i>	1.84±0.35 <i>Bby</i>	1.9±0.27 <i>Bby</i>
Root-plate diameter (cm)	R2	23.9±0.87 <i>Aax</i>	19.3±0.47 <i>Abx</i>	17.7±1.11 <i>Acx</i>	16.5±1.02 <i>Bax</i>	15.7±0.68 <i>Bax</i>	13.4±0.41 <i>Bax</i>
	R6	27.2±1.78 <i>Bay</i>	25.8±1.66 <i>Aay</i>	16.6±0.78 <i>Aby</i>	25.1±0.65 <i>Aay</i>	22.9±0.93 <i>Aay</i>	18.2±0.98 <i>Bby</i>
Total root-plate biomass (g pl^{-1})	R2	3.0±0.17 <i>Aax</i>	3.5±0.27 <i>Aax</i>	2.9±0.26 <i>Aax</i>	1.7±0.21 <i>Bax</i>	1.8±0.24 <i>Bax</i>	1.4±0.15 <i>Bax</i>
	R6	6.4±0.39 <i>Aay</i>	4.4±0.40 <i>Aby</i>	5.0±0.51 <i>Aby</i>	2.9±0.15 <i>Bay</i>	2.1±1.30 <i>Bbx</i>	1.7±0.16 <i>Bbx</i>
Stem-root plate root biomass ratio	R2	4.1±0.47 <i>Bbx</i>	5.4±0.31 <i>Aax</i>	–	5.8±0.19 <i>Aax</i>	5.9±0.14 <i>Aax</i>	–
	R6	4.9±0.39 <i>Bay</i>	5.5±0.61 <i>Bax</i>	–	6.4±0.76 <i>Aay</i>	6.9±0.79 <i>Aay</i>	–

Values are means±s.e. of three replicates. Different italic upper-case letters indicate significant differences ($P < 0.05$) among genotypes within each CPD and development stage; italic lower-case letters indicate significant differences between CPDs within each genotype and development stage; block lower-case letters indicate significant differences between OS within each genotype and CPD

Table 3 Root-plate root biomass (g plate-layer⁻¹) for genotypes CF29 and Zenit in the 0–5 cm (1st) and >5 cm (2nd) layers of the root-plate, at the R2 and R6 development stages (floral bud andend of anthesis, respectively), and for three CPDs (d1: 5.6; d2: 10 and d3: 16 plants m⁻², respectively) Data from Exp. 1

Development stage	Root-plate layer	CF29 Crop population density			Zenit Crop population density		
		d1	d2	d3	d1	d2	d3
R2	1st	1.97±0.21 <i>Aax</i>	2.44±0.46 <i>Aax</i>	1.95±0.29 <i>Aax</i>	0.82±0.21 <i>Aax</i>	0.96±0.36 <i>Aax</i>	0.58±0.14 <i>Aax</i>
	2nd	1.05±0.13 <i>Bax</i>	1.07±0.07 <i>Bax</i>	1.00±0.22 <i>Bax</i>	0.90±0.21 <i>Aax</i>	0.86±0.11 <i>Aax</i>	0.83±0.15 <i>Aax</i>
R6	1st	4.24±0.50 <i>Aay</i>	4.03±0.72 <i>Aay</i>	3.93±0.80 <i>Aay</i>	2.14±0.29 <i>Aay</i>	0.42±0.07 <i>Aay</i>	0.56±0.22 <i>Aax</i>
	2nd	1.79±0.14 <i>Bay</i>	1.64±0.18 <i>Bby</i>	1.30±0.27 <i>Bbx</i>	1.12±0.16 <i>Bax</i>	0.35±0.07 <i>Bby</i>	0.31±0.05 <i>Bby</i>

Values are means ± s.e. of three replicates. Different italic upper-case letters indicate significant differences ($P<0.05$) between layers within each genotype and CPD; italic lower-case letters indicate significant differences between CPD and genotype; and block lower-case letters indicate significant differences between OS within each root-plate layer, genotype and CPD

population density on biomass in layer 2 (>5 cm) for both genotypes. In this layer and for both genotypes, plants grown at higher crop population densities had lower root biomass: a reduction (with respect to d1) of 27.4 % for CF29 as 72.3 % for Zenit was observed (Table 2). There was a significant OS * CPD interaction ($P<0.05$) in Layer 2 of root-plate biomass (Table 1), that was related to the different patterns of response of this variable to CPD at the R2 and R6 stages (Table 3). The effect of OS was significant, with a few exceptions, both at the whole root-plate biomass level (Table 2) and in each of the layers (Table 3).

Stem-root plate root biomass ratio

Differences between genotypes for stem-root plate root biomass ratio were only evident at d1 in both the R2 and the R6 stages, with Zenit showing the largest ratio ($P<0.001$; Table 2). Effects of CPD on this variable were limited to CF29 at the R2 OS stage. The effect of OS was significant ($P<0.05$; Table 1) and the stem-root plate root biomass ratio was higher at R6 for both genotypes, except for CF29 at d2 (Table 2).

Number of roots in the root-plate

At both ontogenetic stages there was an effect of genotype on root-plate root numbers, with CF29 exhibiting, at both the R2 and R6 stages, greater root numbers than Zenit in each of the three diameter categories ($P<0.05$, Fig. 1a and b). The greatest difference was at the level of fine roots (0–1 mm), about 2.6 times more numerous in CF29 at both OS. Both genotypes had 10 times more

fine roots (0–1 mm) than in the other diametrical categories (Fig. 1a and b). Increasing crop population density did not cause significant changes in root number ($P>0.05$) for either genotype. There was an effect of OS on the number of roots of the three diameter categories at each genotype (Table 1).

Total length of root-plate roots

At both ontogenetic stages and in the two root-plate layers the root total length across diameter classes, root-plate layers and development stages were much greater in CF29. There was a predominance of the 0–1 mm diameter class contribution to total length in both layers, and a greater root length in the upper layer of the root-plate (Fig. 2) and these effects were almost always significant at $P<0.05$. As the crop developed (from the R2 to the R6 OS), the differences between genotypes were maintained, especially in the first layer, with a greater length for CF29 (Fig. 2). Increasing crop population density from d1 to d3 had a negative effect ($P<0.05$) on the root lengths of both genotypes, especially in the roots of the second layer. At the greatest CPD and in the second layer the differences between genotypes became very small indeed ($P>0.05$). The effect of OS on this variable was significant for the finest roots (0–1, 1.1–2 mm), while for those roots with >2 mm of diameter size there was no effect of OS (Table 1).

Root-plate root axial breakage force

There was no effect of crop population density (CPD: $P=0.16$) on root-plate root axial breakage force. Data for

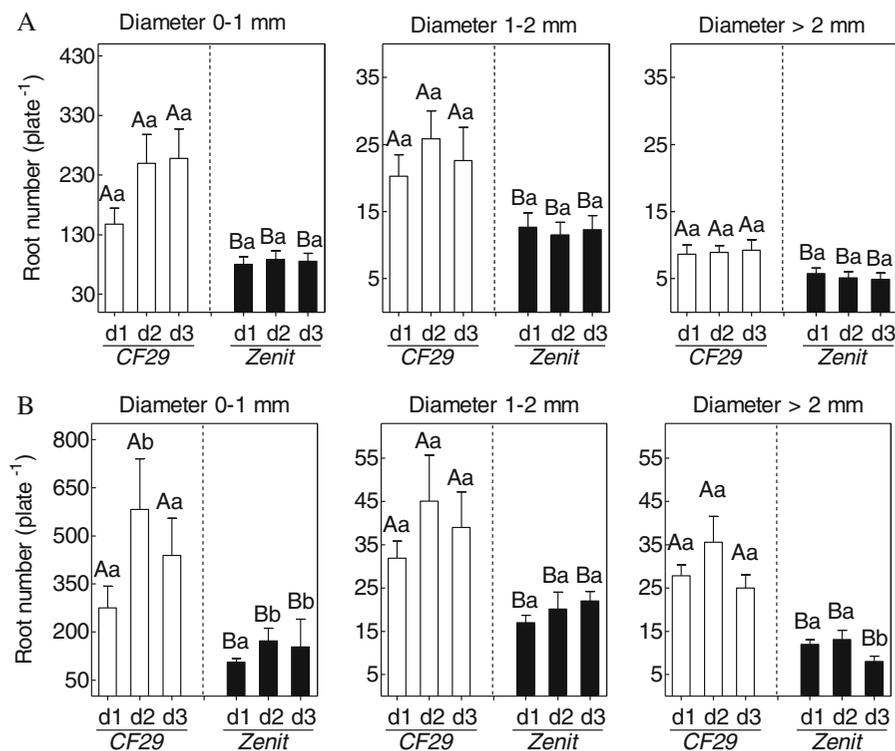


Fig. 1 Root number per root-plate grouped by diameter categories, for genotypes CF29 and Zenit grown at three crop population densities (d1: 5.6; d2: 10; d3: 16 plants m⁻²) at the **a** R2 (floral bud 0.5 to 2 cm from insertion of uppermost leaf stage); and **b** R6 (end of anthesis) developmental stages. Different *uppercase letters* indicate significant differences ($P < 0.05$) among genotypes within

each root diameter category, and different *lower case letters* indicate significant differences between CPD's within each genotype. Values are means \pm s.e. of three replicates. Note the changes in scales between the left and remaining panels in both **a** and **b** and between all panels in **a** and **b**

different CPDs and developmental stages were pooled, and the analysis focused on the differences between genotypes. These analyses showed that root axial breakage force was linearly and positively correlated with the diameter of the root in both genotypes (Fig. 3), and in both cases these correlations were significant. The slope of this relationship was significantly greater in CF29 (slope test: $P < 0.0001$), and root axial breakage force was significantly higher in CF29 for all roots of a diameter greater than 1.5 mm ($P < 0.01$, Fig. 3).

Discussion

Our results show linkages between root-plate root morphological and mechanical properties and root lodging tolerance (as reflected in variations in *rfm* and root-plate

diameter) across genotypes, crop population densities and developmental stages. Relationships between root-plate root properties, such as root number, root length, root biomass and root axial breakage force, with the force required to induce root lodging across ranges of crop population densities and crop ontogenetic stages were established. To the best of our knowledge, this is the first report for an annual crop where these issues have been explored in an integral way. In both experiments the results relating to the effects of genotype, crop population density and development stage on root failure moment (*rfm*) and root-plate diameter were consistent with those of Sposaro et al. (2008), thus confirming the value of our experimental model. In addition, the fact that the results of both experiments were consistent, across years, in relation to the above variables, and in relation to total root-plate root biomass provides a strong

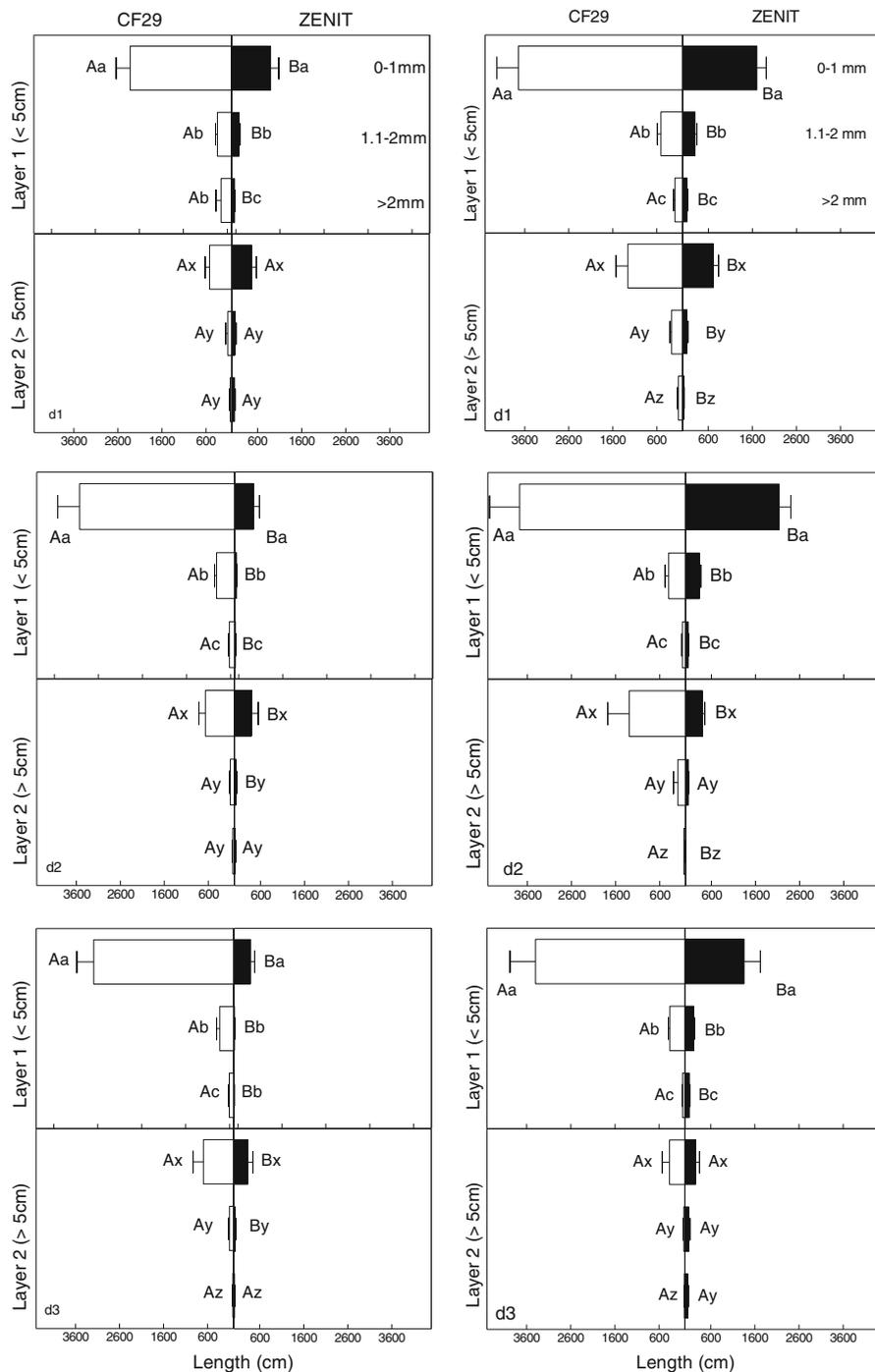


Fig. 2 Total root-plate root length (cm) for genotypes CF29 and Zenit in the two layers of the root-plate (0–5 and >5 cm) for the three crop population densities (d1: 5.6; d2: 10; y d3: 16 plants m⁻²); at the **a** R2 (floral bud 0.5 to 2 cm from insertion of uppermost leaf) and **b** R6 (end of anthesis) stages. Different upper

case letters indicate significant differences ($P < 0.05$) between the genotypes within each root diameter category, and different lower case letters indicate significant differences between root diameter categories within each genotype and CPD. Values are means \pm s.e. of three replicates

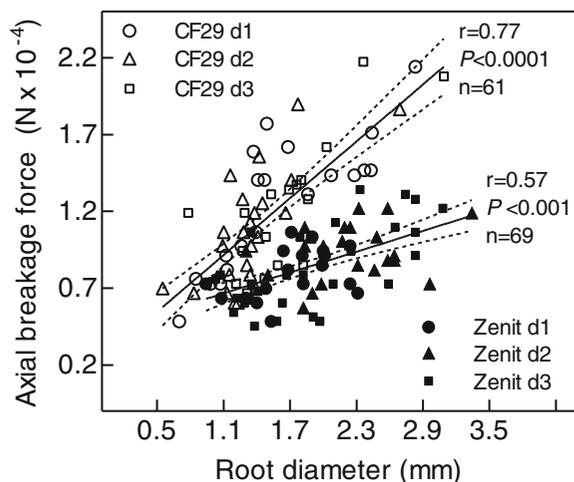


Fig. 3 Relationships between root axial breakage force ($N \times 10^{-4}$) and root diameter for genotypes CF29 and Zenit, grown at three crop densities (d1: 5.6; d2: 10; y d3: 16 plants m^{-2}). Data for measurements made at the R2 and R6 development stages (Schneider and Miller 1981) and from both layers (1 and 2) were pooled. Fitted functions were $y=0.61x+0.24$ ($r=0.77$, $P<0.0001$) for CF29 and $y=0.23x+0.41$ ($r=0.57$, $P<0.001$) for Zenit. Dashed lines indicate the 95 % confidence intervals for the fitted functions

degree of confidence that the effects of the main factors on the stem-root plate biomass ratio (variable only measured in Exp. 2) were likely to have been replicated in Exp 1. Previous work by Sposaro et al. (2010) has explored the environmental controls of root lodging in sunflower at the whole plant level and in some detail. Root failure moment was estimated as a function of root-plate diameter and soil texture properties (eq. 2 in Sposaro et al. 2010).

Biomass allocation and root-lodging tolerance in sunflower

The ability of tap-root system related with anchorage function is linked to the mass of the root-plate in tree species (Couts 1986). However, the information about the shoot: root allometry of plants is scarce respect to anchorage function of the root systems (Ennos 1993). Our results for shoot: root-plate root biomass ratios (Tables 1 and 2) are the first to explore these issues in herbaceous plants and in relation to genotype differences in root-lodging tolerance. Loades et al. (2010) and Burylo et al. (2012) have examined these issues in herbaceous species but in relation to strength of the soil colonised by roots rather than root-lodging tolerance.

Properties of root-plate roots and genotype differences in root-lodging tolerance

Works in other species have shown that the number and length of the roots in the root-plate, among other attributes of the anchorage system, are responsible for the ability of the plant to resist forces that might cause root lodging (El-Khouly 1995; Mickovski and Ennos 2003; Wu 1995; Wu et al. 1988). In this sense, the more resistant plants would be those with a high density of fine roots (1–2 mm) in the superficial layers and larger diameter roots (>2 mm) in the deeper layers (Reubens et al. 2007). In the case of sunflower, the root lodging tolerant genotype showed a greater number of fine roots compared to thicker roots and a higher total number of lateral roots (Fig. 1a and b). As shown for genotype CF29 as well as for other species (Ennos 1989) a greater number of fine roots in the more superficial layers of the root-plate seem to be related with a high tolerance to root lodging. For some cereals, such as wheat (Baker et al. 1998) and barley (Scott et al. 2005), anchoring efficiency of plants is positively associated with the total length of roots in the root-plate. The notion that increasing root biomass distributed in the top few centimetres of the root-soil hemisphere would provide greater anchorage had only been demonstrated in sunflower seedlings (Ennos 1989, 1993). In the case of adult sunflower plants growing under field conditions, we found that the tolerant genotype (CF29) had similar characteristics to those described above, showing a greater root length and greater fine root biomass (0–1 mm) in the first layer (0–5 cm) of the root-plate. Together, these features help to explain the higher tolerance of CF29 to root lodging as compared to Zenit.

Root axial breakage force is related to root diameter but is also dependent on genotype

The effect of root diameter on its mechanical properties (“size effect”) have recently been studied in woody species (Bischetti et al. 2005; De Baets et al. 2008; Genet et al. 2005, 2008), shrubs (De Baets et al. 2008; Tossi 2007), and in grasses (De Baets et al. 2008; Loades et al. 2010, 2013; Mattia et al. 2005). All of them are reported as root tensile strength (which is curvilinearly related to diameter), while our results are expressed as axial breakage force (which is linearly related to diameter; Fig. 3). We decided to show the axial breakage force as a linear function of the root

diameter because this allows a clear distinction of genotype differences in mechanical properties of roots, and the statistical analyses required to compare these fitted functions are simpler and more powerful. Despite the differences in the way the mechanical properties of roots are shown, both approaches are comparable. We have transformed root tensile strength values (taken from the above-mentioned papers) into root axial breakage forces. Both sunflower genotypes showed steeper axial breakage force/diameter relationships than grasses, but these were less steep than those found in woody and shrub species (data not shown). Ours is the first record for a crop species in which the mechanical properties of individual root-plate roots has been studied in relation to the root lodging process across ontogenetic stages and over a range of crop population densities (CPD). Our results also show that the root axial breakage force/diameter relationship is genotype-dependent (Fig. 3), something which has not been explored in other species. This finding raises questions about the possible effects of these genotype differences on root-plate root mechanical properties and on the anatomical origins of these differences. This idea is supported by our results, since for equal diameter size the axial breakage force is different between genotypes, suggesting that the anatomical properties of roots are different, as secondary walls of xylem vessels, suberin and lignin deposition, number and size of xylem vessels, among others.

Increases in CPD affects root-plate root properties

The effect of increased crop density on different attributes of the root system had been studied in other species of agronomic interest. An example is *Festuca rubra* where a reduction in the number and total root biomass with increasing crop density was observed (Pechackova 1999). For wheat it was found that the total length of the roots was negatively affected by crop density, weakening plant anchorage (Sparkes et al. 2008). Other studies for barley, wheat and maize, showed that an increase in crop population density, resulted in increased competition between plants, affecting the total plant biomass and making them more vulnerable to root lodging (Berry et al. 2000; Dupuy et al. 2004; Easson et al. 1993, 1995; Hébert et al. 2001; Scott et al. 2005). For sunflower, a reduction in root failure moment with increasing crop population density was reported by Sposaro et al. (2008). Our results reinforce this information and show that higher crop

population densities reduced the root-plate diameter (Table 2) and decreased the total length (Fig. 2) and biomass of roots especially in layer 2 (>5 cm; Table 3), and at the highest crop densities (10 and 16 plants m⁻²). These results are in line with other studies (e.g., *Festuca rubra*, Pechackova 1999; wheat, Berry et al. 2000).

Between R2 and R6, and in both genotypes, an increase in the number of fine roots (0–1 mm diameter) and a decrease in the root length were observed (Fig. 1a and b) at the plant density of 10 plants m⁻², (Fig. 2a and b). It is known that the availability of resources has a greater effect on the number of roots than on their length (Fitter et al. 1991). On this basis it is reasonable to think that the individual growth of the roots (not the number) was affected negatively with increasing competition for resources caused by the increase in the crop density. In natural conditions, other species such as grasses can change their root morphology with increasing competition for resources (McConnaughay and Bazzaz 1991).

Changes in root-plate root properties during crop development

Crop ontogenetic stage also affects root system attributes related to root lodging. In rice (*Oryza sativa* L.) it was shown that the number of adventitious roots increased with the progress of the crop developmental stages, and that this variable was positively related to the total force necessary to lodge the plant (Oladokun and Ennos 2006). In sunflower, with the progress of the crop cycle from R2 to R6, root failure moment increased in both genotypes (Table 2; Sposaro et al. 2008). Our results show that extensive root-plate variables, such as root-plate diameter, root-plate biomass, root number (Fig. 1) and root length (Fig. 2) all increased with the progress of the crop cycle (from R2 to R6; Table 2) in both genotypes (tolerant or susceptible to root lodging). These changes relate to the increased root lodging failure moment observed in R6 with respect to R2, especially in the tolerant genotype (CF29; Table 2).

Conclusions

Our results provide new information about sunflower root-plate root morphology and mechanical properties, their relationship with root-lodging tolerance, and their responses to CPD and crop development. Taken together, they suggest that variations in root-lodging tolerance

in response to the principal factors studied in our experiments arise from the action of a number of root-plate root traits (root-plate root length, root-plate root number, root-plate root biomass and root-plate root axial breakage force). Modelling (see Sterling et al. 2003; Stokes et al. 1996 for examples) of root lodging tolerance as affected by these traits and further experimentation and analyses are necessary to determine the relative importance of each trait in conferring root lodging tolerance in sunflower. Our results also underline the need to examine the anatomical properties of root tissues underlying the genotype differences shown in Fig. 3, such as cell wall thickness, lignin and suberin depositions in secondary walls, among others.

Acknowledgments We thank Advanta Semillas SAIC for access to seed of the hybrids used in the experiments, and Gustavo Striker for helpful comments about this manuscript. This research was supported by grants from UBACyT (UBA G048) and FONCyT (PICT 13159). M.E. Manzur was supported by FONCyT (Fund for Scientific and Technological Research) scholarships and AJH is member of CONICET, the National Research Council of Argentina. We thank two anonymous reviewers for their thoughtful comments on the previous version of this report.

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