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Effects of ethephon on anatomical changes in sunflower (*Helianthus annuus* L.) stems associated with lodging

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Abstract. Stem lodging causes significant losses in crops of cereals and oilseeds. The aim of the present study was to identify the anatomical causes that generate differences in response to stem lodging in sunflower. Two sunflower hybrids (Stay-Green, resistant to stem lodging; Zenit, susceptible to stem lodging) were grown at three crop population densities and artificially lodged at two advanced ontogeny stages (R7 and R8), which were preceded by ethephon application near the flower button stage (R1). Measurements included stem failure moment of force (*Bs*), thickness of primary and secondary structures in the stem lodging zone (*t*), diameter of the stem lodging zone (*di*), sclerenchyma packages area (*sp*), secondary xylem tissue area (*xt*) and yield. Stay-Green had significantly higher values for *Bs*, *t*, *di*, *sp* and *xt*. At higher crop densities and more advanced ontogeny stages these parameters were reduced, favouring stem lodging, although the effects were ameliorated by ethephon application through anatomical modifications. Zenit exhibited the greatest responses to ethephon application. The present study is the first field study identifying anatomical changes causing stem lodging and intraspecific variability in sunflowers. The information provided can be used by geneticists in selection programs for stem lodging tolerance in the context of increasing crop population densities to improve sunflower yield.

Additional keywords: ethylene, tolerance to lodging.

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Introduction

Yield losses in various cereal and oilseeds crops due to root or stem lodging can be very important. In Argentina, the crops most affected by lodging are maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.); approximately 10% of the sunflower crop area lodges annually, representing an estimated loss of US$40 million (Bragachini *et al.* 2001). Sunflower cultivation area in Argentina extends from the south-east of the Pampas to the region of the Chaco. Expansion of the agricultural frontier of soybean displaced sunflower crops to more marginal areas exposed to adverse environmental conditions, where the risk of lodging is higher, thereby causing losses in yield.

Stem lodging is defined as a fracture that usually occurs in the internodes of the lower one-third of the plant (Pinthus 1974; Baker *et al*. 1998). This event can be described as the result of the force of the wind acting on the upper sections of the plant that bend the plant at its base. The stem breaks when the force moment reaches a critical level (i.e. critical load) that exceeds the lodging stem moment (Berry *et al*. 2004). The occurrence of lodging can depend on the genotype, crop population density and crop developmental stage, because these variables together can affect the mechanical and structural properties of the plant (Hall *et al.* 2010). Sposaro *et al*. (2008) conducted a detailed study on the effects of these factors on root lodging. Observations by breeders tend to agree that sunflower is most susceptible to stem lodging between flowering and harvest maturity (A. de la Vega, pers. comm.). A study on the effects of source–sink relationships on the force required to produce stem breakage also indicated a reduction in this force between flowering and harvest maturity (Polack 1992). Differences between genotypes in susceptibility to stem lodging have been proven for other species, like pea (Beeck *et al.* 2006), wheat, barley (Crook and Ennos 1994; Berry *et al.* 2003*a*, 2003*b*, 2006; Kelbert *et al.* 2004*a*) and *Miscanthus* (Kaack and Schwarz 2001). In sunflower, there is evidence of intraspecific variability in susceptibility to stem lodging (Hall *et al.* 2010). High crop population densities are expected to raise thinner, and presumably weaker, stems, which can favour lodging. The effects of crop population density and crop

developmental stage have been little explored in relation to susceptibility to stem lodging, although the effects of genotype have received some attention for the aforementioned species. In sunflower, studies have been fewer and far less systematic. Studies on stem lodging in several species (e.g. wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), pea (*Pisum sativum*), *Miscanthus giganteus* and *Miscanthus sinensis*) have shown that the thickness of the wall of the hollow, approximately cylindrical stems is more closely related to lodging susceptibility than stem diameter (e.g. Crook and Ennos 1994; Kaack and Schwarz 2001; Berry *et al.* 2003*a*, 2003*b*, 2006; Kelbert *et al.* 2004*b*). Because, in contrast, the stems of sunflowers are solid, even though pith parenchyma cells tend to lose turgor and the pith tissue becomes friable as development proceeds, some anatomical differences in the traits defining tolerance to lodging can be also expected. In the present study, we measured the thickness of primary and secondary structures in the stem lodging zone (*t*), stem diameter in the lodging zone (*di*), sclerenchyma package area (*sp*) and secondary xylem tissue area (*xt*) in stems in the lodging zone to determine the importance of these variables in conferring stem lodging tolerance.

In sunflower, studies using foliar applications of plant growth regulators have shown changes in morphology and yield (Spitzer *et al*. 2011; Koutroubas *et al*. 2014; Koutroubas and Damalas 2015). Ethephon (2-chloroethylphosphonic acid; Ethrel-480; Bayer CropScience) is a growth regulator that acts by releasing ethylene within the plant following spontaneous decomposition. In sugarcane, exogenous applications of ethephon produced changes in anatomical parameters of the stem, including the thickness of phloem, the diameter of fibrovascular bundles and the diameter of parenchyma cells (Marrero *et al*. 2005).

Stem lodging tolerance has been studied in oats (*Avena sativa* L.; Pinthus 1974), wheat (Pinthus 1974; Crook and Ennos 1994; Baker *et al*. 1998; Berry *et al.* 2003*a*, 2003*b*, 2007; Kelbert *et al*. 2004*a*, 2004*b*), barley (Pinthus 1974; Dunn and Briggs 1989; Berry *et al.* 2006) and rice (*Oryza sativa* L.; Zhang *et al.* 2014, 2016), with the results describing properties of the stem and its tissues, such as lignin and cellulose, the size of the xylem vessels and allometric relationships related to plants with different degrees of stem lodging (Dunn and Briggs 1989; Kelbert *et al*. 2004*a*). In wheat and barley, it was found that the association between the length and diameter of the stem and characteristics of stem tissues confer mechanical properties that determinate the degree of stem lodging (Mulder 1954; Berry *et al*. 2006, 2007). A high content of lignin and cellulose in stem tissues is positively correlated with high rigidity (Kaack *et al*. 2003). Ramos and Herrera (2013) demonstrated that application of ethephon stimulated lignin deposition in *Pinus radiata*. Studies in sorghum (*Sorghum bicolor*) crops revealed that hybrids possessing the character 'stay green' or delayed senescence have greater resistance to stem lodging than the genotype with 'fast dry down' or accelerated senescence (Rosenow 1984; Thomas and Howarth 2000). Hall *et al*. (2010) demonstrated that 'stay green' hybrids perform better to deal with stem lodging than 'non-stay green' genotypes at increasing crop densities.

Because current sunflower hybrids, when protected from lodging and disease, show increases in yield potential at crop population densities up to almost threefold the common density of 5 plants m^{-2} used nowadays (López Pereira *et al.* 2004), it seems very likely that the propensity for lodging at high crop densities also plays a part in reducing realisable yield potential. Thus, identifying the anatomical characteristics that cause differences in the degree of stem lodging and to establish some simple relationships between stem morphology at the breakage site and stem failure moment of force could help breeders seeking to improve cultivar tolerance to stem lodging in the context of increasing crop population densities with the aim of improving sunflower yield.

The present study is the first field study that compares, within the same species, two materials with different degrees of susceptibility to stem lodging and analyses, from an anatomical point of view, the anatomical features underlying these differences. It is also the first study to investigate the anatomical changes that occur as a result of ethephon application that can increase tolerance to stem lodging in high-density sunflower crops.

Materials and methods

Experimental design and crop growth conditions

The experiment was performed on a silty clay loam soil (Typic Argiudoll) at the Faculty of Agronomy, University of Buenos Aires (FAUBA; 34°35′S, 58°29′W). The soil tillage was direct seeding. Two hybrids of contrasting susceptibility to stem lodging were used (Advanta V70597, resistant to stem lodging (Advanta Semillas); and Zenit, susceptible to stem lodging (Sursem)). Putative ranking for hybrid susceptibility to stem lodging was provided by A. de la Vega (pers. comm.). Advanta V70597 is a stay-green (SG) hybrid with canopies senescing more slowly during grain filling and after physiological maturity than the Zenit hybrid, the canopies of which exhibit a fast dry down syndrome over these developmental phases. Crops were sown by hand in rows spaced at 0.7 m, and thinned at the V4 stage (Schneiter and Miller 1981) to a density of 5.6 plants m^{-2} (low density: LD), 10 plants m^{-2} (medium density: MD) and 16 plants m^{-2} (high density: HD). A randomised complete block design with three replicates was used, with five treatments per hybrid: LD (5.6 plants m^{-2}), MD, MDE (10 plants m^{-2} plus ethephon (E) application), HD and HDE (16 plants m^{-2} plus ethephon application). The size of each plot was nine rows (including two border rows) by 6 m length, and there were five randomised plots per block. Ethephon (Ethrel-480; Bayer CropScience) was sprayed on the leaves near the flower button stage (R1; the terminal bud forms a miniature floral head rather than a cluster of leaves and, when viewed from directly above, the immature bracts exhibit a many-pointed star-like appearance; Schneiter and Miller 1981) at a concentration of 0.75 L ha^{-1} (Merrien 1998) in the MDE and HDE treatments. There was no application of ethephon to LD plants because lodging tolerance at low density is more likely related to intraspecific variability between genotypes; in addition, producers do not apply any plant growth regulator for plants planted at low density. In contrast, crop density is expected to have an effect on the degree of tolerance to stem lodging at MD or HD plantings (Hall *et al.* 2010). Measurements were made at stages R7 and R8 (R7, the back of the head has started to turn a

pale yellow colour and the grain reaches 50% of final dry weight; R8, the back of the head is yellow but the bracts remain green and the grain has reached 90% of its final dry weight; Schneiter and Miller 1981), when sunflower is most susceptible to stem lodging (A. de la Vega, pers. comm.).

Crops were grown without water or nutritional restrictions, and insects and diseases were controlled throughout the experimental period.

Measurement of stem failure moment of lodging at the breaking point

Plants were artificially lodged at the R7 and R8 developmental stages using specialised equipment, as described by Sposaro *et al*. (2008), basically consisting of a horizontal push-bar attached to two vertical arms that had pivots anchored to the soil on the axis of the row section. Measurements were only performed when the surface soil was sufficiently dry as to provide the plants with firm anchorage to avoid root lodging. After removal of the leaves and the capitulum, the instrument push-bar was set at 60% of plant height (*h*) in contact with the stem. In preliminary experiments using the lodging instrument, we found that if the push-bar was set higher than 70% of total *h*, stem lodging did not occur because the upper portion of the stem is flexible and the push-bar slips over it. Therefore, we chose to set the push-bar at 60% of total *h*. Force was applied to the push-bar at the point where it came into contact with the stem by means of steel cable attached to a windlass and pulley system linked to a balance (Model HEC; Balanzas Electronicas Torres), and the stem was displaced in 58 steps from its vertical position until stem breakage occurred. At the end of each incremental displacement, the force at the balance (*FB*; kg), the angle between the cable and the push-bar (αP) and the angle of displacement of the stem from the vertical were registered (see Sposaro *et al*. 2008). When the stem broke, the unbroken stem closest to the point of breakage was sectioned vertically and the thickness of primary and secondary structures in the stem lodging zone (*t*; effective stem wall thickness) and that of the pith was measured. The height of the breakage point from the soil surface and the diameter of the stem at that point were also measured. Forces registered at the balance at each incremental step were transformed, using standard decomposition of forces procedures, to estimate the force (*FP*; in N) acting perpendicular to the stem as follows:

$$FP = FB \times \cos \alpha, \text{ where } \alpha = 90 - \alpha P \qquad (1)$$

and the highest FP obtained in each run was taken to be the force needed to induce stem lodging for the plant. The stem failure moment of force (*Bs*; N m), the moment of force needed to induce stem lodging, was obtained by multiplying *FP* (N) by 0.6*h* (m).

Anatomical tissues in the fracture zone

Tissues samples from the stem breaking zone were taken by cutting a longitudinal portion of approximately 0.5 cm that included the rupture site. This material was set in FAA (10% formaldehyde, 47.5% ethanol, 37.5% water, 5% glacial acetic acid) and immediately embedded in paraffin according to the methods described by Johansen (1940). Using a Minot rotary microtome, transverse sections (12–15 μm) were cut and double stained using safranin and fast green. This allowed identification of primary and secondary walls, mainly *xt* and *sp* (Conn *et al*. 1960). The histological sections were analysed under a Zeiss-Axioplan optical microscope and photographs were taken with a digital camera (Canon Power Shot G9).

From the digital images obtained, the area of the primary and secondary walls was determined using image processing software (Adobe Photoshop 10; Adobe Systems Software).

Total plant dry weight

At the R9 stage, when the bracts become yellow and brown (this stage is regarded as physiological maturity; Schneiter and Miller 1981), five plants per treatment and genotype combination were harvested and separated into leaves, stem, capitulum and petioles. These organs were oven dried over 5 days at 80°C and then weighed.

Grain yield

At the R9 stage (Schneiter and Miller 1981), four capitulums were harvested for both hybrids and in all five treatments groups. In each capitulum, the number of filled grains and dry weight per grain were determined (Chimenti *et al*. 2002). In the MD and HD treatments, plants were staked to avoid stem lodging that would have prevented the harvesting of capitulums.

Statistical analysis

Four-way analysis of variance (ANOVA) was performed using hybrid (H), ontogeny stage (S), crop population densities (D) and ethephon (E) as predictor variables in InfoStat V. 2014I (Di Rienzo *et al*. 2010). Whenever interactions were significant, simple effects were evaluated, and Tukey's test was performed for comparisons between treatments. The number of replicates was three and the level of significance was set at 5%. Principal components analyses (PCA) were performed using H, S, D, E, *t*, *di*, *sp* and *xt* as the main variables in InfoStat V. 2014I (Di Rienzo *et al*. 2010).

Results

Stem failure moment of force

Significant differences were found for *Bs* between hybrids, crop population densities, ethephon application and ontogeny stages ($P < 0.05$; Table 1). With advancing ontogeny stage, there was a significant decrease in *Bs* ($P < 0.05$) for both hybrids at the three crop densities, regardless of ethephon application. The S × D interaction was significant ($P < 0.05$), reflecting that differences between ontogeny stages varied with density (Table 1).

At the R7 stage, the hybrid SG had significantly higher *Bs* values than the Zenit hybrid at all crop densities, regardless of ethephon application ($P < 0.05$; Fig. 1*a*).

At the R7 and R8 ontogeny stages, *Bs* values in both hybrids were lower as crop density increased, regardless of ethephon application. This reduction in *Bs* values was significant ($P < 0.05$) between the LD and other treatments for the SG hybrid. For the Zenit hybrid, there was a significant ($P < 0.05$) reduction between the LD and other treatments without ethephon application at the R7 stage, but not at the R8 stage (Fig. 1).

Table 1. Summary analysis of variance (ANOVA) table for the effects of hybrid (H), ontogeny stage (S), crop population densities (D), ethephon (E) and their interactions on stem failure moment of force (*Bs*), thickness of primary and secondary structures in the stem lodging zone (*t*), diameter of the stem lodging zone (*di*), sclerenchyma packages area (*sp*) and secondary xylem tissue area (*xt*)

n.s., Not significant

Source of variation	*F*-value					*P*-value				
	Bs	*t*	*di*	*sp*	*xt*	*Bs*	*t*	*di*	*sp*	*xt*
H	173.55	2.52	141.7	803.4	126.47	<0.05	n.s.	<0.05	<0.05	<0.05
S	261.74	234.3	117.3	595.8	2184.67	<0.05	<0.05	<0.05	<0.05	<0.05
D	48.2	54.17	122.1	401	287.45	<0.05	<0.05	<0.05	<0.05	<0.05
E	19.49	16.31	65.77	345.7	473.99	<0.05	<0.05	<0.05	<0.05	<0.05
H × S	48.19	25.18	2.54	285.6	1.87	<0.05	<0.05	n.s.	<0.05	n.s.
H × D	12.75	43.45	46.2	41.91	100.42	<0.05	<0.05	<0.05	<0.05	<0.05
H × E	1.19	1.53	0.02	5.03	2.09	n.s.	n.s.	n.s.	<0.05	n.s.
S × D	6.17	1.56	41.66	42.84	79.95	<0.05	n.s.	<0.05	<0.05	<0.05
S × E	0.51	0	0.51	10.99	0.06	n.s.	n.s.	n.s.	<0.05	n.s.
D × E	0.01	0.16	0.21	0.2	0.13	n.s.	n.s.	n.s.	n.s.	n.s.
H × S × D	0.66	14.66	9.92	82.04	2.91	n.s.	<0.05	<0.05	<0.05	n.s.
H × S × E	0.25	0.09	0.32	16.08	8.4	n.s.	n.s.	n.s.	<0.05	<0.05
H × D × E	0.02	0.05	0	0.29	0.28	n.s.	n.s.	n.s.	n.s.	n.s.
S × D × E	0.36	0.32	16.53	6.76	6.06	n.s.	n.s.	<0.05	<0.05	<0.05
H × S × D × E	0.07	0.25	0.01	0.07	0.3	n.s.	n.s.	n.s.	n.s.	n.s.

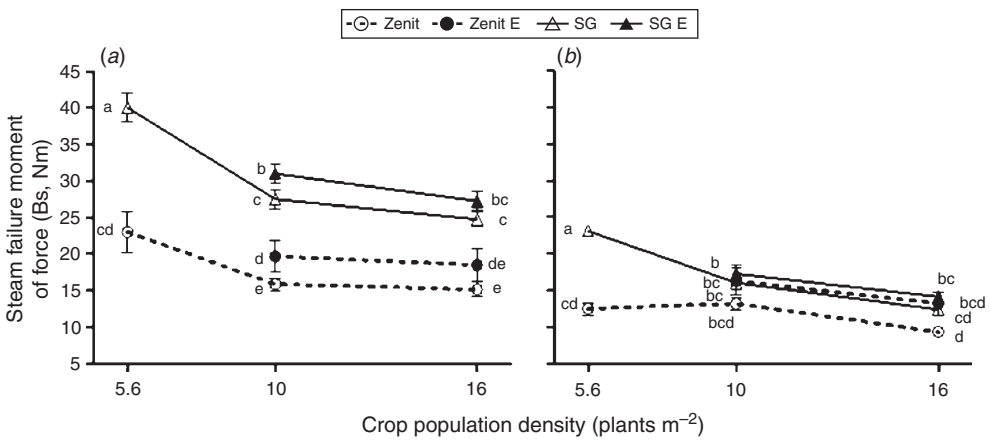

Fig. 1. Stem failure moment of force (*Bs*) measured at the (*a*) R7 and (*b*) R8 stages (Schneiter and Miller 1981) for the two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem), grown at three crop population densities (low (5.6 plants m^{-2}), medium (10 plants m^{-2}) and high (16 plants m^{-2}), with (E) and without application of ethephon. Data are the mean ± s.e.m. of three replicates. Different letters next to symbols indicate significant ($P < 0.05$) differences across hybrids, crop population densities and application of ethephon.

For both hybrids, ethephon application resulted in an increase in *Bs* at each ontogeny stage and for each planting density (Fig. 1). This increase in *Bs* was equivalent to 11.4% at the R7 and R8 stages for the SG hybrid at MD and HD, and to 22.8% and 32.7% at the R7 and R8 stages respectively for the Zenit hybrid. So, the Zenit hybrid showed greater responses to ethephon application than the SG hybrid (Fig. 1).

Thickness of primary and secondary structures in the stem lodging zone

Significant differences ($P < 0.05$) were found in *t* values between crop population densities, ethephon application and ontogeny stages. The significant H × S × D interaction ($P < 0.05$) indicated that the differences between hybrids varied with ontogeny across crop densities (Table 1).

Both increases in crop density and advancing ontogeny reduced *t* values. For both hybrids, these reductions were statistically significant ($P < 0.05$) at the R7 and R8 stages only in the LD planting (Fig. 2). This reduction in *t* values was significant ($P < 0.05$) between the LD planting and the remaining treatments for the SG hybrid. The Zenit hybrid exhibited a significant ($P < 0.05$) reduction between the LD planting and treatments without ethephon application at the R7 stage, but not at the R8 stage (Fig. 2).

Ethephon applications always resulted in an increase in *t* values for both hybrids, in both the MD and HD plantings and at the R7 and R8 stages (Fig. 2). This increase in *t* values was

equivalent to 8.1% and 20.6% at the R7 and R8 stages respectively for the SG hybrid at both densities (MD and HD), and increased to 23% at the R7 and R8 for the Zenit hybrid.

Stem lodging zone diameter

Significant differences ($P < 0.05$) were found in *di* values between hybrids, crop population densities, ethephon application and ontogeny stage. Significant interactions ($P < 0.05$) were found for H × S × D and S × D × E (Table 1).

Increasing crop density and advancing ontogeny stage reduced *di* values in both hybrids. At the R7 stage, these reductions were statistically significant ($P < 0.05$) between LD and the other treatments for both hybrids, whereas at the R8 stage the reductions in *di* were significant ($P < 0.05$) at increasing crop density for the SG but not Zenit hybrid (Fig. 3). The *di* values were always higher in the SG than Zenit hybrid (Fig. 3).

Ethephon application always resulted in an increase in *di* values (in both hybrids, at both MD and HD plantings and for both ontogeny stages). For the Zenit hybrid the increase was equivalent to 10.75% in both MD and HD at the R7 stage and to 12.4% at the R8 stage; for the SG hybrid, *di* values increased by 8.71% and 13.92% at the R7 and R8 stages respectively (Fig. 3).

Sclerenchyma packages area

Significant differences ($P < 0.05$) were found for *sp* values between hybrids, crop densities, ethephon application and

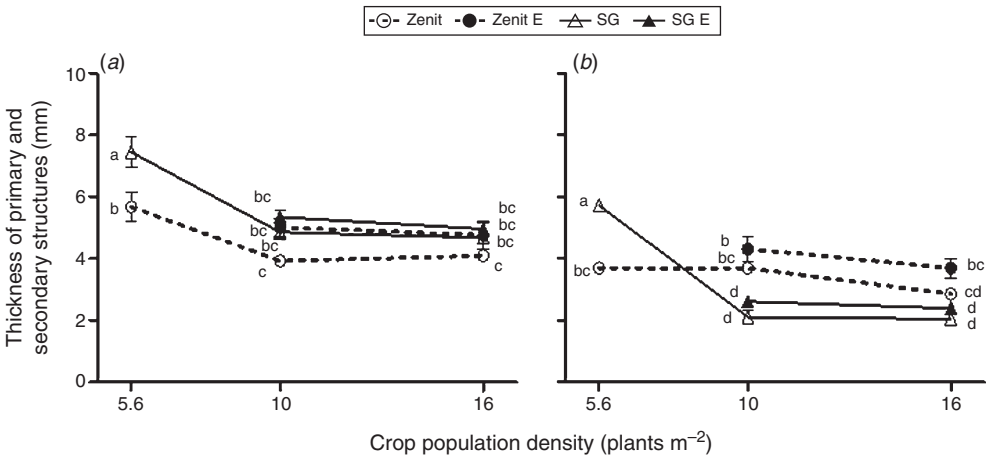

Fig. 2. Thickness of primary and start of secondary structures measured at the (*a*) R7 and (*b*) R8 stages (Schneiter and Miller 1981) in two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem), grown at three crop population densities (low (5.6 plants m^{-2}), medium (10 plants m^{-2}) and high (16 plants m^{-2}), with (E) and without application of ethephon. Data are the mean ± s.e.m. of three replicates. Different letters next to symbols indicate significant ($P < 0.05$) differences across hybrids, crop population densities and application of ethephon.

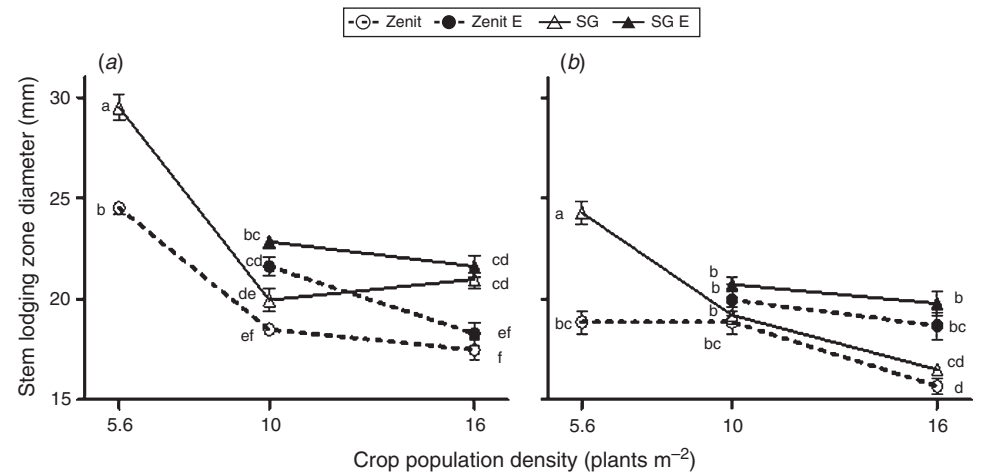

Fig. 3. Stem lodging zone diameter measured at the (*a*) R7 and (*b*) R8 stages (Schneiter and Miller 1981; scale) in two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem), grown at three crop population densities (low (5.6 plants m^{-2}), medium (10 plants m^{-2}) and high (16 plants m^{-2}), with and without application of ethephon. Data are the mean ± s.e.m. of three replicates. Different letters next to symbols indicate significant ($P < 0.05$) differences across hybrids, crop population densities and application of ethephon.

ontogeny stage. Significant interactions ($P < 0.05$) were found for H × S × D, H × S × E and S × D × E (Table 1).

Changes in ontogeny stage and crop densities reduced *sp* values (Figs 4, 5). These reductions were significant ($P < 0.05$) between LD and MD at the R7 stage for both hybrids, and at the R8 stage only for the SG hybrid (Fig. 5). Conversely, the Zenit hybrid showed a significant ($P < 0.05$) reduction in *sp* with an increase in crop density at the R8 stage. The reduction in *sp* values in the LD planting between the R7 and R8 stages was equivalent to 42.2% and 3.7% for the SG and Zenit hybrids respectively (Fig. 5).

The SG hybrid at the R7 stage had higher ($P < 0.05$) *sp* values than the Zenit hybrid at all crop densities, regardless of ethephon application (Fig. 5*a*). At the R8 stage, there were no significant differences between hybrids at LD and MD plantings, regardless of ethephon application; however, at the HD planting, *sp* values were higher ($P < 0.05$) for the SG than Zenit hybrid (Fig. 5*b*).

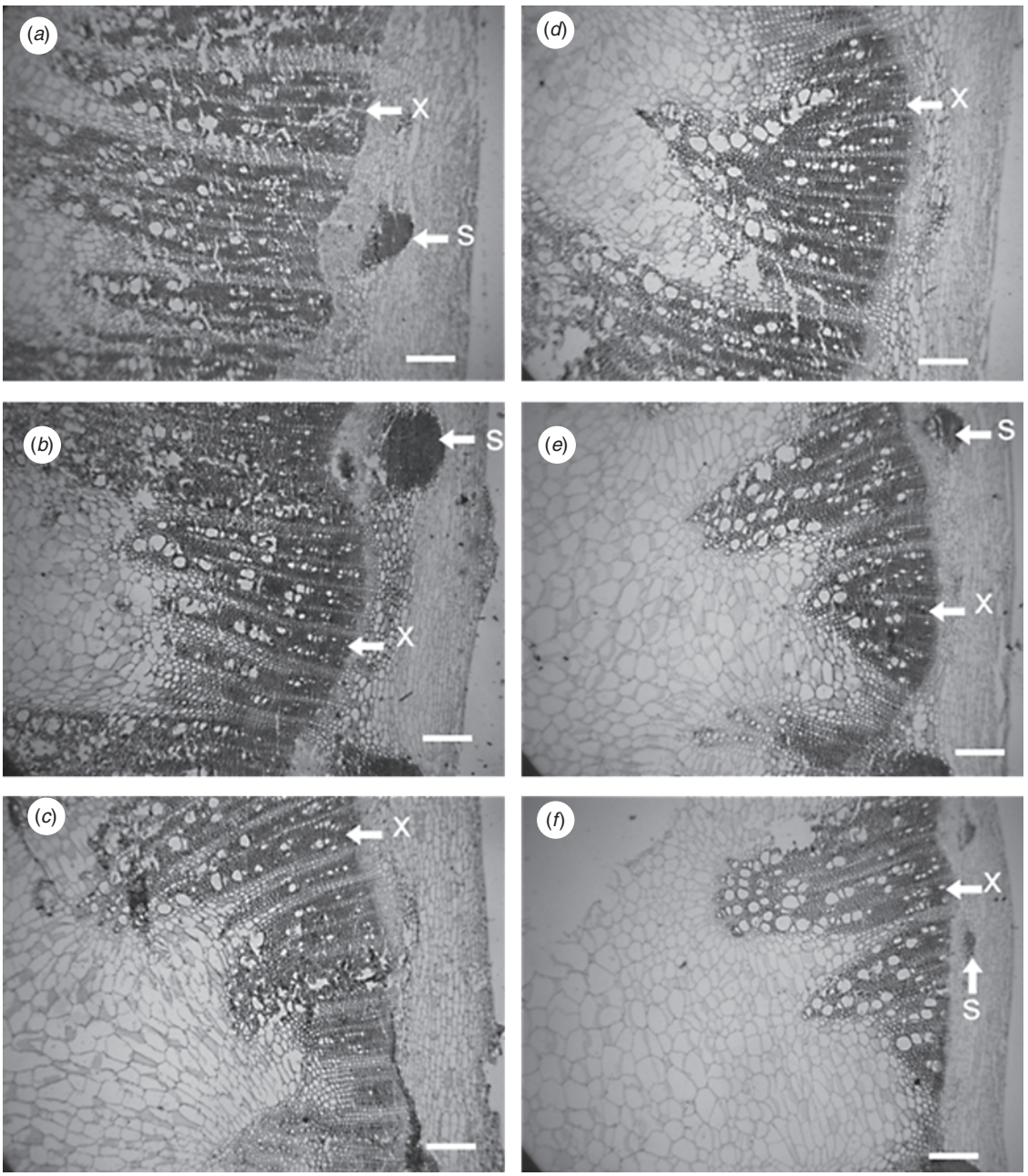

Fig. 4. Xylem and sclerenchyma packages in the stem lodging zone of the sunflower hybrid Advanta V70597 (stay-green; SG) at the (*a*–*c*) R7 and (*d*–*f*) R8 stages without ethephon application for three crop population densities: (*a*, *d*) low density (5.6 plants m^{-2}), (*b*, *e*) medium density (10 plants m^{-2}) and (*c*, *f*) high density (16 plants m^{-2}). X, xylem; S, sclerenchyma packages. Scale bars = 334 µm.

Ethephon application reduced the decrease in *sp* values in both hybrids, at the R7 and R8 stages and with crop density (Fig. 5). These reductions were equivalent to 13.15% and 41.8% at the R7 and R8 stages respectively for the SG hybrid and 25% at the R7 and R8 stages for the Zenit hybrid compared with no ethephon application.

Secondary xylem tissue area

Significant differences were found ($P < 0.05$) for *xt* values between hybrids, crop population densities, ethephon application and ontogeny stage. Significant interactions ($P < 0.05$) were found for H × S × E and S × D × E (Table 1).

Advances in crop ontogeny (from R7 to R8) reduced *xt* values (Figs 4, 6). Under LD planting, *xt* values were reduced by 38.3% and 49% in the SG and Zenit hybrids respectively (Fig. 6).

Increases in crop population density (MD and HD) reduced *xt* values (Figs 4, 6). For the SG hybrid, these reductions were significant ($P < 0.05$) at the R7 and R8 stages. For the Zenit hybrid, the reductions were significant ($P < 0.05$) for LD versus MD at the R7 stage and for LD versus HD at the R8 stage (Fig. 6). For SG hybrid plants that were not treated with ethephon, 33.6% and 42.3% reductions in *xt* were observed at MD and HD plantings compared with LD at the R7 stage. At the R8 stage, the reductions in *xt* were 31.3% and 42.3% for MD and HD

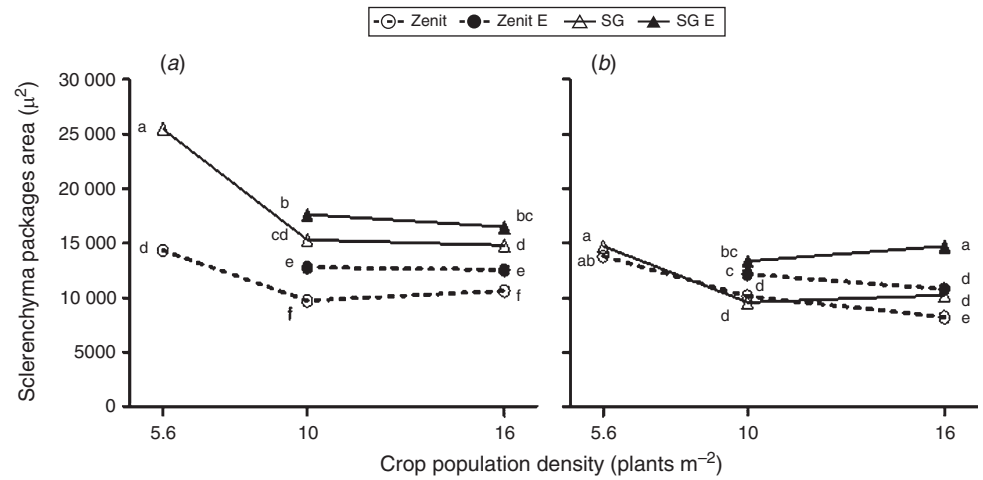

Fig. 5. Sclerenchyma packages area per section observed at the (*a*) R7 and (*b*) R8 stages (Schneiter and Miller 1981; scale) in two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem), grown at three crop population densities (low (5.6 plants m^{-2}), medium (10 plants m^{-2}) and high (16 plants m^{-2}), with (E) and without application of ethephon. Data are the mean ± s.e.m. of three replicates. Different letters next to symbols indicate significant ($P < 0.05$) differences across hybrids, crop population densities and application of ethephon.

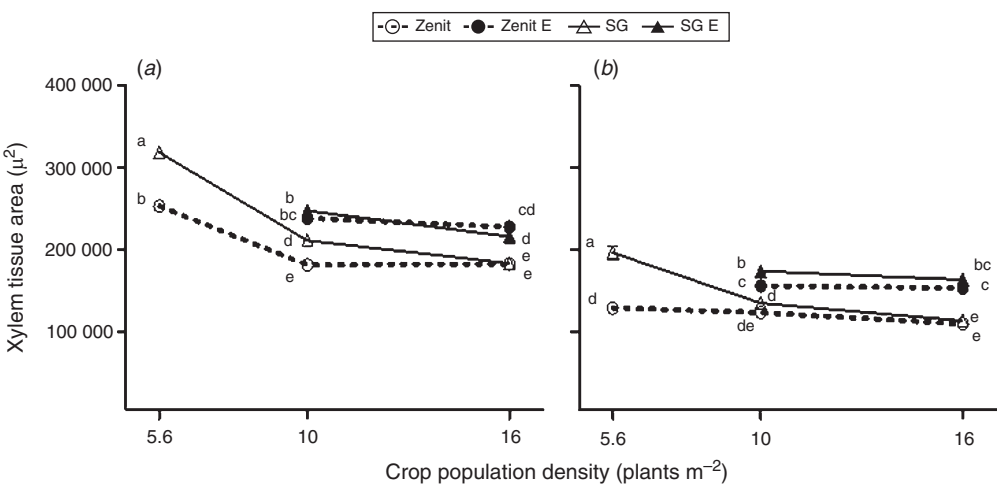

Fig. 6. Xylem tissue area at the (*a*) R7 and (*b*) R8 stages (Schneiter and Miller 1981; scale) in two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem), grown at three crop population densities (low (5.6 plants m^{-2}), medium (10 plants m^{-2}) and high (16 plants m^{-2}), with (E) and without application of ethephon. Data are the mean ± s.e.m. of three replicates. Different letters next to symbols indicate significant ($P < 0.05$) differences across hybrids, crop population densities and application of ethephon.

respectively versus LD (Fig. 6). For the Zenit hybrid, the reductions compared with LD were 28.2% and 27.8% for MD and HD respectively at the R7 stage and equivalent to 4.3% and15.4% respectively at the R8 stage (Fig. 6).

Ethephon application ameliorated the decreases in *xt* values in both hybrids across ontogeny stages and crop densities (Fig. 6). This amelioration in the reduction of *xt* values was significant ($P < 0.05$) at the R7 stage for both hybrids and densities (MD and HD), whereas at the R8 stage it was significant for the SG hybrid at both densities only. Conversely, at the R8 stage, there was a significant ($P < 0.05$) increase in *xt* at MD and HD compared with LD for the Zenit hybrid (Fig. 6).

Importantly, the declines observed in *xt* with advancing crop development could be associated with the fact that stem lodging occurs at higher heights at the R8 stage. The results showed a significant ($P < 0.05$) linear relationship between the area of the secondary xylem tissue and *Bs* (Fig. 7).

Principal component analysis

Excluding ethephon treatment from the analysis

Axis 1 of the PCA explained 61.9% of the total variability of data. Such variability was composed of the following variables (in order of importance): area corresponding to the secondary xylem tissue ($r = 0.96$), diameter stem lodging zone ($r = 0.92$), sclerenchyma packages area ($r = 0.91$), thickness of primary and secondary structures in the stem lodging zone ($r = 0.88$), hybrid ($r = 0.36$), planting density ($r = –0.60$) and ontogeny stage ($r = –0.61$; see Fig. 8).

Axis 2 of the PCA explained 15.8% of variability and was composed of the following variables: ontogeny stage ($r = 0.70$), hybrid ($r = 0.63$), sclerenchyma packages area ($r = 0.18$), diameter stem lodging zone ($r = 0.18$), thickness of primary and secondary structures in the stem lodging zone ($r = –0.16$), area corresponding to the secondary xylem tissue ($r = –0.18$) and planting density ($r = –0.32$; Fig. 8).

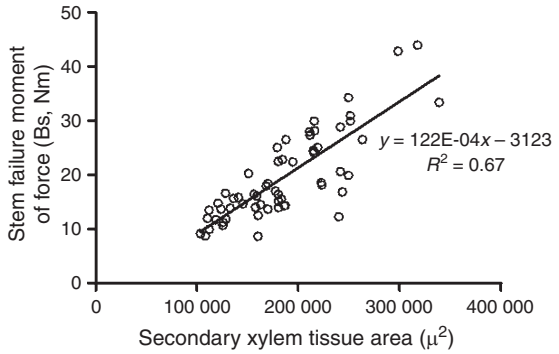

Fig. 7. Relationship between stem failure moment of force (*Bs*) and xylem tissue area for all treatments (crop population densities of 5.6, 10 and 16 plants m^{-2}), ontogeny stages (R7 and R8; Schneiter and Miller 1981) and ethephon application for the two hybrids (Advanta V70597 (stay-green) and Zenit). The relationship was linear and significant ($P < 0.05$; $n = 3$).

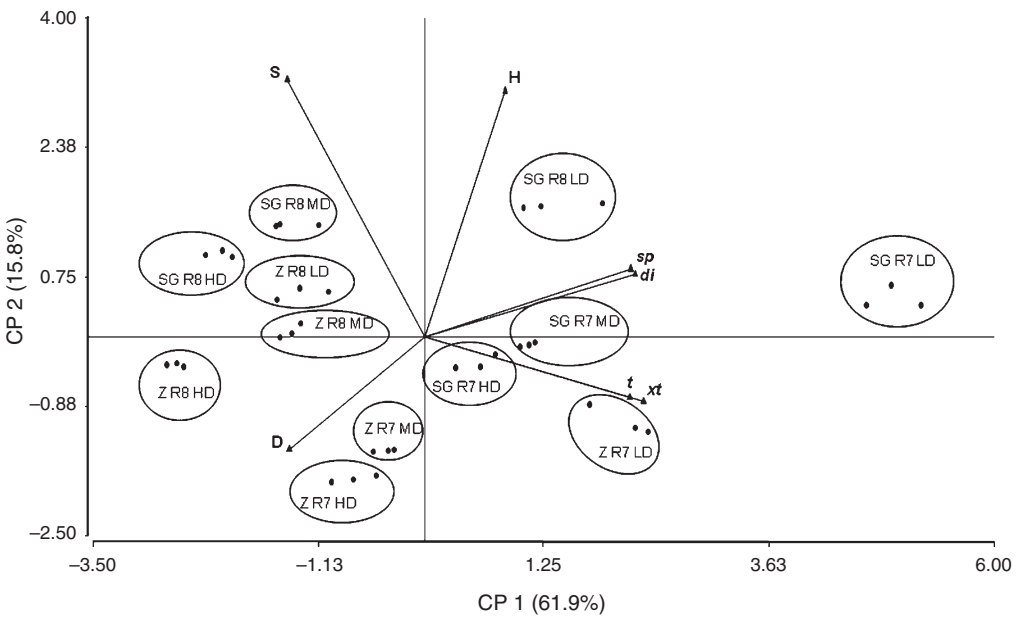

Fig. 8. Principal component analysis excluding ethephon treatment of two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem; Z), grown at three crop population densities (5.6, 10 and 16 plants m^{-2} = LD, MD and HD respectively), without application of ethephon. Data points represent the mean of each treatment for each block ($n = 3$). Hybrid (H), ontogeny stage (S), crop population densities (D), thickness of primary and secondary structures in the stem lodging zone (*t*), diameter of the stem lodging zone (*di*), sclerenchyma packages area (*sp*) and secondary xylem tissue area (*xt*) were the variables (triangles). PC, principal component.

Including all treatments in the analysis

Axis 1 of the PCA explained 50.1% of the total variability, which was composed of the following variables: area corresponding to the secondary xylem tissue ($r = 0.94$), sclerenchyma packages area ($r = 0.88$), diameter stem lodging zone ($r = 0.87$), thickness of primary and secondary structures in the stem lodging zone ($r = 0.82$), hybrid ($r = 0.34$), ethephon ($r = 0.04$), planting density ($r = –0.49$) and ontogeny stage ($r = –0.65$; see Fig. 9).

Axis 2 of the PCA explained 16.7% of variability. Main variables in order of importance were: ethephon ($r = 0.78$), densities ($r = 0.72$), area corresponding to the secondary xylem tissue ($r = 0.20$), thickness of primary and secondary structures in the stem lodging zone ($r = 0.06$), sclerenchyma packages area ($r = –0.02$), hybrid ($r = –0.05$), diameter stem lodging zone ($r = –0.13$) and ontogeny stage ($r = –0.37$; Fig. 9).

Grain yield

Grain yield increased for both hybrids ($P < 0.05$) at increasing planting density (Fig. 10) from LD to HD. For the Zenit hybrid, yield was improved by 10% when crop density increased from LD to MD in plants that had not been treated with ethephon and by 14% in plants treated with ethephon. Compared with the LD planting, yield increased by 56% and 66% at the HD planting without and with ethephon application respectively. For the SG hybrid, yield increased by 14% when crop density increased from LD to MD in untreated plants, and by 26% in plants sprayed with ethephon. Finally, grain yield increased by 74% and 78% in HD versus LD for untreated and ethephon-treated plants respectively (Fig. 10).

Carbohydrate partition

Ethephon application resulted in a trend towards higher values for the dry weight of stems and petioles, in particular at high planting densities (data not shown).

Discussion

The differences found in the anatomical characters between the two hybrids explain the intraspecific variability in stem lodging tolerance in sunflowers.

Changes in the thickness of the primary and secondary structures at the stem lodging zone caused by increases in crop density and/or related to advances in crop ontogeny between the SG and Zenit hybrids explain the differences in stem lodging tolerance between the hybrids in favour of the lodging-tolerant SG hybrid. Kaack *et al*. (2003), working with 17 different hybrids of *Miscanthus* spp. found similar results, where the largest percentage of these tissues increased the modulus of elasticity with the consequent effect of decreasing risk of stem lodging due to lower stem deformation (Figs 1, 2).

A considerable part of the thickness of primary and secondary structures at the stem lodging zone consists of cellulose and lignin depositions (data not shown), so that modifications in this characteristic correspond to progressive increases in these two components from the R7 to R8 stage and/or changes in crop density, whereby lower amounts of these components are expected to be found at higher crop densities, leading to thinner individual stems. Increases in both components confer tolerance to stem lodging. The results of the present study are in agreement with those of Dunn and Briggs (1989) and Kelbert *et al*. (2004*a*), who studied barley and wheat and found

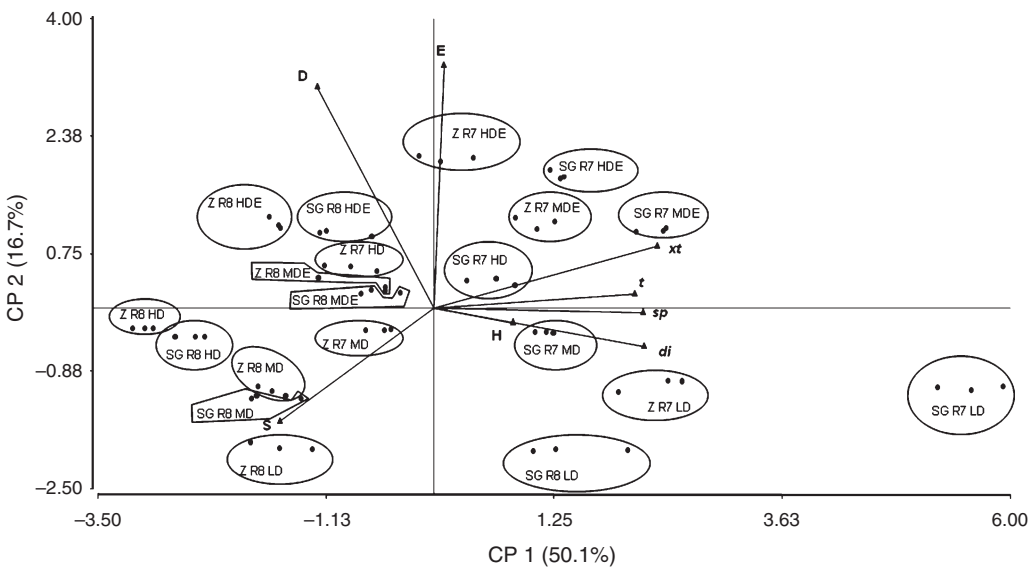

Fig. 9. Full principal component analysis of two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem; Z), grown at three crop population densities (5.6, 10 and 16 plants m^{-2} = LD, MD and HD respectively), with and without application of ethephon (+E and –E respectively). Data points represent the mean of each treatment for each block ($n = 3$). Hybrid (H), ontogeny stage (S), crop population densities (D), ethephon (E), thickness of primary and secondary structures in the stem lodging zone (*t*), diameter of the stem lodging zone (*di*), sclerenchyma packages area (*sp*) and secondary xylem tissue area (*xt*) were the variables (triangles). PC, principal component.

associations between the length and diameter of the stem and characteristics of stem tissues conferring mechanical strength to stems to prevent lodging. There was a positive correlation between *Bs* values and the thickness of primary and secondary structures at the stem lodging zone for both genotypes (SG and Zenit; Fig. 11), suggesting that cellulose and lignin play an important role in conferring rigidity to stems, as also found by Kaack *et al*. (2003) in *Miscanthus* hybrids.

Changes in the diameter at the stem lodging zone, which affects the degree of lodging tolerance across crop densities and developmental stages, could be explained by either: (1) remobilisation (reductions between the R7 and R8 stages); or (2) less translocation of carbohydrates (in case of changes in crop densities) to the stem. The presence of an active photosynthetic area during the grain-filling period can help restrict these processes. The Zenit hybrid examined in the present study is a 'fast dry down' material, with accelerated leaf senescence, so that strong remobilisation of photoassimilates from the stem can be expected to sustain effective grain filling. This behaviour is thought to be the cause of the decrease in stem diameter in Zenit plants, leading to reduced tolerance to stem lodging (Fig. 3). Polack (1992) showed that, in sunflowers, variations in the source–sink relationship during the grain-filling period can modify stem diameter, thereby increasing susceptibility to stem lodging. In that study, Polack (1992) removed (or not) capitulum (the main carbohydrate sink) to restrain (or not) carbohydrate remobilisation to grains, noting that lower remobilisation was related to greater stem diameter, and therefore a greater force was needed to provoke stem failure.

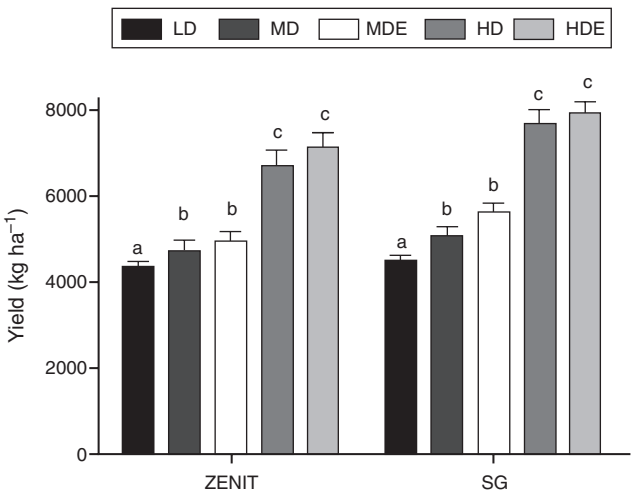

Fig. 10. Yield of two sunflower hybrids, namely Advanta V70597 (stay-green; SG) and Zenit (Sursem), grown at three crop population densities (5.6, 10 and 16 plants m^{-2} = LD, MD and HD respectively), with (E) and without application of ethephon. Data are the mean ± s.e.m. (n = 3). Within each hybrid, columns with different letters differ significantly (P < 0.05).

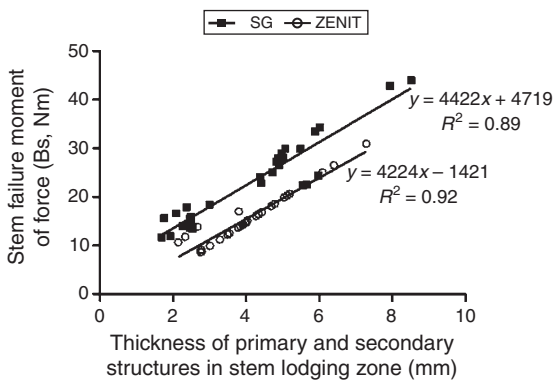

Fig. 11. Relationships between stem failure moment of force (*Bs*) and thickness of primary and secondary structures in the stem lodging zone for the Advanta V70597 (stay-green; SG) and Zenit (Sursem) hybrids grown at three crop population densities (5.6, 10 and 16 plants m^{-2}) at two ontogeny stages (R7 and R8; Schneiter and Miller 1981), with or without ethephon application. The relationships for each hybrid were linear and significant (P < 0.05; n = 3).

Secondary xylem tissue is also an important trait that helps prevent stem lodging. The results of the present study indicate that higher secondary xylem tissue values conferred high performance to deal with stem lodging. The SG hybrid had higher values for secondary xylem tissue than the Zenit hybrid (Fig. 6), and these findings are in line with those reported by Kelbert *et al*. (2004*b*) in wheat, where cultivars with a higher percentage of secondary xylem tissue had significantly higher tolerance to stem lodging.

Analysing the behaviour of stem area associated with sclerenchyma packages per stem section revealed differences between the two hybrids during both stages of the ontogeny cycle (R7 and R8 stages). The SG hybrid had higher amounts of sclerenchyma, which confers a higher degree of stem lodging tolerance at both stages, as well as at increasing crop densities, on this hybrid (Fig. 5). We clearly showed a significant positive linear relationship between the area of the sclerenchyma packages and *Bs* (Fig. 12). In this regard, the results of the present study are supported by those of Dunn and Briggs (1989), who screened barley crops and found a positive relationship between the area of sclerenchyma packages and resistance to stem lodging.

The results of the present study, in which we investigated the anatomical basis of stem lodging in sunflowers by comparing

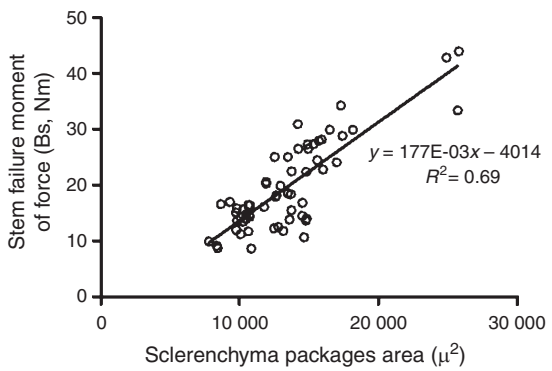

Fig. 12. Relationships between stem failure moment of force (*Bs*) and sclerenchyma packages area per section observed for all treatments (crop population densities 5.6, 10 and 16 plants m^{-2}, ontogeny stages R7 and R8 (Schneiter and Miller 1981) and with or without ethephon application) and for the Advanta V70597 (stay-green; SG) and Zenit (Sursem) hybrids. The relationships were linear and significant (P < 0.05; n = 3).

two hybrids with differences in this character, revealed that three main anatomical characteristics are responsible for stem rigidity, and therefore largely determine stem lodging tolerance: secondary xylem tissue, sclerenchyma packages area and the thickness of primary and secondary structures.

Ethephon application had significant effects in both hybrids, at all stages and at both MD and HD plantings, increasing *Bs* (Fig. 1), so improving stress lodging performance. The positive effects of ethephon on *Bs* values were based on changes detected at the anatomical level, because the secondary xylem tissue, sclerenchyma packages area and thickness of primary and secondary structures increased with ethephon application, and consequently improved tolerance to stem lodging. Koutroubas *et al*. (2014) found that application of plant growth regulators in sunflower improved stem lodging tolerance, but also decreased grain yield. In the present study, ethephon improved stem lodging tolerance but, unlike Koutroubas *et al*. (2014), we did not find any change in grain yield (Fig. 10).

In conclusion, the present study provides the first results for sunflower crops grown under field conditions of the basis of intraspecific differences in the behaviour of hybrids with different degrees of susceptibility to stem lodging, and the effects of crops density and ontogeny on this. We also show, for the first time, that application of ethephon, a plant growth regulator that increases stem lodging tolerance, could be effective in increasing lodging tolerance in sunflower crops under field conditions, and that this agronomic practice can have particular effects on susceptible plants growing at high crop densities. Future research should focus on determining the physiological responses complementing the anatomical determinants identified herein, as well as determining molecular markers that could help in selection programs aimed at obtaining hybrids with a higher degree of tolerance to stem lodging.

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