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Canopy stay-green and yield in non-stressed sunflower

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ABSTRACT

Delayed leaf senescence during the grain filling phase, or stay green (SG), may be functional or cosmetic; the first being considered a valuable trait in breeding of many crop species. To establish whether canopy senescence patterns exhibited by two sunflower (*Helianthus annuus* L.) hybrids visually selected for slow post-anthesis canopy leaf area index (LAI) loss reflected functional, rather than cosmetic, SG, LAI dynamics and total biomass increase between anthesis and physiological maturity were followed in three separate experiments in which the putative SG hybrids were compared with standard (i.e., non stay green, NSG) hybrids exhibiting normal rates of canopy senescence under non- or minimal-water stress conditions. In two experiments, pairwise (i.e., one SG vs. one NSG) comparisons were made at two crop population densities. In the third experiment, grown at a single crop population density, four NSG hybrids were contrasted with the two SG hybrids. Canopy senescence dynamics were well described by fitted bilinear functions which discriminated between an initial, slow phase of leaf area loss and a second phase of rapid canopy senescence. No differences between hybrids in the rate of senescence during the first phase were found, but the putative SG hybrids exhibited a significantly slower rate of senescence during the second phase and a significantly higher LAI at physiological maturity (as % of LAI at anthesis). One NSG hybrid showed a greater rate of second-phase senescence than the remaining three hybrids in this category. Across experiments, the anthesis-physiological maturity increment in total oil-corrected biomass and radiation use efficiency (RUE) were significantly greater in the SG hybrids. Cluster analysis based on these four attributes, using data from Exp. 3, clearly discriminated between SG and NSG hybrids. Examination of specific leaf N (SLN) dynamics suggests that the lower RUE values observed in one of the NSG hybrids could be attributable to lower SLN values, but this was not the case for the remaining NSG hybrids. It is also possible that very rapid canopy LAI loss in another NSG hybrid may underlie its lower RUE. The failure of SG hybrids to translate greater post-anthesis biomass increment into grain yield across experiments was associated with their slightly shorter times to anthesis and their significantly lower biomass at anthesis. Grain number, the component of yield most strongly associated with yield, was associated with biomass at anthesis. A further contributing factor was that the duration of grain-filling tended to be shorter in SG hybrids. In the one experiment in which this variable was measured, resistance to stalk breakage was greater in the SG hybrids than the NSG one to which they were compared. We conclude that the SG observed in the hybrids with slower canopy senescence is functional and not cosmetic, and that for this to be translated into a yield advantage all hybrids need to reach anthesis at the same time and grain-filling duration also has to be the same across hybrids. Stay green is an interesting secondary trait to select for and should lead to higher and more stable yields in environments in which stem breakage (lodging) is a problem.

1. Introduction

Delayed leaf senescence during the grain filling phase of grain crops, or stay green (SG), may be functional, when the loss of canopy capacity for carbon fixation is delayed or occurs at a slower rate, or cosmetic, when maintenance of leaf chlorophyll is combined with the disassembly of the photosynthetic apparatus (Thomas and Smart, 1993; Thomas and Howarth, 2000). Functional SG is con-

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sidered a valuable trait in breeding of many crop species, since it contributes positively to increases in yield potential through increments in biomass production (e.g., wheat, Evans, 1993; maize, Rajcan and Tollenaar, 1999a, 1999b) or yield stability under conditions of water shortage (sorghum, Borrell et al., 2000; *Miscanthus*, Clifton-Brown et al., 2002), late sowings (sunflower, de la Vega and Hall, 2002a, 2002b) or high plant population density (maize, Duvick, 1997; Tollenaar and Wu, 1999).

Functional stay green can also increase resistance to stalk breakage (stem lodging) by preventing (or minimizing) remobilisation of carbohydrates from the stem during grain filling by maintaining crop photosynthesis (Schneiter et al., 1987). Experiments in which sunflower crops were protected against diseases and lodging demonstrated that grain yield increased with crop population density well beyond the commercial density used in Argentina (López Pereira et al., 2004). Susceptibility to stem and root lodging in sunflower (as in other crops) increases with crop population density (Sposaro et al., 2008; Hall et al., 2010), suggesting that stay green could be a valuable secondary trait in selection for higher and more stable yields in this crop species.

Cukadar-Olmedo and Miller (1997) and Cukadar-Olmedo et al. (1997) studied the variability and inheritance of stem colour at maturity in sunflower (which they termed stay green). The stay-green referred in the present work relates to maintenance of green leaves in the canopy, in tune with the current usage of the term used to refer to this attribute (e.g., Thomas and Howarth, 2000).

The dynamics of specific leaf N (SLN, $\mathrm{g\,N\,m^{-2}}$ leaf) occupies a central position in the issue of functional stay green, since leaves are an important source of N for grain filling while, on the other hand, maintenance of leaf photosynthesis in SG cultivars could support the continued absorption of N by the roots (Borrell et al., 2001; van Oosterom et al., 2010a, 2010b). High SLN (or leaf N content, $\mathrm{g\,N\,kg^{-1}}$) at anthesis has been linked to delayed leaf senescence in sorghum and maize (Borrell and Hammer, 2000; He et al., 2005; van Oosterom et al., 2010b). In experiments using standard sunflower hybrids conducted to examine the effects of N supply on sunflower mean canopy SLN (senescent leaves excluded), SLN has been found to either fall fairly continuously during the season (Bange et al., 1997), or to remain fairly stable for some 35 days during the middle of the season and fall rather sharply as from mid-grain fill (Hall et al., 1995). The relationship between maximum (at light saturation) photosynthetic capacity and SLN for individual sunflower leaves is well established (Connor et al., 1993; Trápani and Hall, 1996; Bange et al., 1997). By contrast, the relationship between crop radiation use efficiency (RUE, g biomass MJ intercepted $\mathrm{radiation^{-1}}$) and SLN does not behave as predicted from theoretical approaches based on single-leaf properties, especially at the low levels of SLN that are typical of the second half of the grain filling phase (Hall et al., 1995; Bange et al., 1997; Massignam et al., 2009). While the causes of this disconnect are still unresolved, there are good arguments to support the concept that SLN must be a highly important component in the determination of RUE.

Here we report the results of experiments designed to test whether the stay green observed in two putative SG hybrids (i.e., hybrids which showed a marked and clearly visible tendency to retain a number of green leaves during the grain filling phase) was functional and not cosmetic (Thomas and Smart, 1993; Thomas and Howarth, 2000). To do this we contrasted several attributes of these putative SG hybrids with those of one or more standard (i.e., NSG, as reflected in their pattern of canopy senescence) hybrids during grain filling. Previous research (de la Vega and Hall, 2002a,b) has shown that the degree of exhibited stay green was strongly influenced by adaptation. Thus, hybrids adapted to the Central region of sunflower cropping in Argentina, when sown late in the region to which they were adapted, showed accelerated canopy senescence, while those adapted to the Northern region did not. An important distinction between the current work and previous research is that all the hybrids used in the current experiments were adapted to the Central region of Argentina and were planted at close to the optimum sowing dates. In some of the experiments we also followed SLN dynamics during grain filling and attempted to establish the relationship between SG and resistance to stalk breakage. The overall objective was to determine if the visual differences in leaf senescence rates are associated with differences in the determinants of oil-corrected grain yield during post-anthesis and exploitable variability for resistance to stalk breakage that could contribute to the formulation of ideotype-based selection strategies to improve yield potential, yield stability or both.

2. Materials and methods

2.1. Genetic materials and field experiments

On the basis of visual observations of sunflower trials conducted across the Central sunflower growing region of Argentina by Advanta Semillas, a set of 6 single-cross hybrids varying in rate of leaf senescence during grain filling were identified. This set includes commercial hybrids widely planted in the Central region, i.e., CF 19 (Advanta Semillas) and Paraíso 20 (Nidera), and experimental hybrids developed by the sunflower breeding program of Advanta Semillas that showed different patterns of leaf senescence. All selected hybrids are intermediate to intermediate-late materials and possess genetic resistance to *Verticillium dahliae* Klebahn, a widespread disease that has the potential to affect yield determinants post-anthesis and confound the results of crop physiological experiments (Sadras et al., 2000). Two of the experimental hybrids (V70597 and V82222) had delayed canopy senescence and were considered as putative functional SG candidates.

The experiments were conducted on a deep coarse loam soil (Typic Hapludoll) at the Advanta Semillas Research Centre, Venado Tuerto, Argentina (33° 41′ S, 61° 57′ W) during the spring-summer seasons of 2000/01 (Exp. 1), 2001/02 (Exp. 2), and 2003/04 (Exp. 3). Daily rainfall and temperature were recorded at a meteorological station sited about 500 m from the experimental plots; and daily incident radiation ($\mathrm{MJ\,m^{-2}\,d^{-1}}$) was estimated from sunshine hours (Ångström, 1924; Prescott, 1940), measured at a meteorological station 1 km from the plots, and estimated radiation receipt above the atmosphere, using the relationship developed by de la Vega and Hall (2002a).Hybrid Paraíso 20 (NSG) was compared with hybrid V70597 (candidate SG) in 2000/01 (Exp. 1) and with V82222 (candidate SG) in 2001/02 (Exp. 2). The experimental design in these two experiments was a factorial combination of two hybrids by two crop population densities ($\delta_1 = 4.51$ plants $\mathrm{m^{-2}}$, $\delta_2 = 6.90$ plants $\mathrm{m^{-2}}$). In 2003/04, the complete set of 6 hybrids (i.e., V70597 and V82222 [both candidate SG], plus Paraíso 20, CF 19, V70740, and V91705 [all four NSG]) was grown at a crop population density of 4.51 plants $\mathrm{m^{-2}}$. In all three experiments, the treatments were arranged in a randomised complete block design with three replicates. Plot size was 8 rows × 6 m with an inter-row spacing of 0.70 m. Planting dates were Exp. 1: 11 October 2000; Exp. 2: 18 October 2001; Exp. 3: 2 October 2003. Tillage practices were conventional and did not vary across years. Nutrient deficiencies were prevented with pre-planting fertilisation. Weeds and insect pests were controlled chemically. Measurements were concentrated on the grain filling phase, using leaf area index (LAI) and total biomass at anthesis as integrative measures of crop growth during the pre-anthesis phase.

2.2. Crop development

Crop phenology was followed from emergence to physiological maturity (PM). Anthesis (A) was defined as the time when 50%

of the plants reached R-5.5 on the Schneiter and Miller (1981) scale. Physiological maturity (time at which maximum grain weight is achieved) was determined from the dynamics of grain growth (Ploschuk and Hall, 1995). To do this, grains from the intermediate portion of the capitulum were harvested every 3–4 days. At each harvest, 3 randomly selected plants per plot that had not been sampled in previous occasions were used, and 5 grains per plant harvested. The grains were dried at 70 °C for at least 48 h before weighing. SigmaPlot (SPSS Science, 2000) was used to fit piecewise bi-linear regression models to individual grain weight/time relationships to estimate the rate and duration of grain filling. A conditional model (Ploschuk and Hall, 1995) was used with a first stage where:

$$\text{Grain weight} = a + b\,\text{DAA}$$

for DAA < C, and a second stage where:

$$\text{Grain weight} = a + b\,C$$

where a and b are the intercept and the slope, respectively, of the linear regression corresponding to the first stage, DAA is days after anthesis, and the constant C is the unknown breakpoint of the function indicating the end of grain filling, which was considered the moment at which PM was reached. Mean r^2 for fitted bi-linear regressions was 0.93, and individual functions had r^2 values ranging from 0.87 to 0.98.

2.3. Above-ground biomass dynamics

Dynamics of aboveground dry matter accumulation was followed from pre-A to past-PM by taking samples of two adjacent plants per experimental unit every week. The plants were separated into organs, and stems were split length-wise into 4 sections to hasten drying. Plant material was dried in a closed glasshouse at 50–60 °C (day) and 40 °C (night) for more than 5 days before weighing. The plant material was extracted from the glasshouse for weighing around noon on hot sunny days only. This technique was tested through the daily tracking of biomass samples weighed before and after oven-drying at 80 °C. No differences in weight were observed from the 4th day of greenhouse drying onwards. In a crop with oil-rich grain such as sunflower, genotypic and environmental effects on seed oil content may confound the interpretation of trial results expressed as biomass. Consequently, crop biomass in post-anthesis and grain yield measurements were corrected for energy expended in oil synthesis using the production values given by Penning de Vries et al. (1983) and assuming that the biomass of non-grain organs contained 2.5% lipids. After discounting a mass of lipid equivalent to 2.5% of the non-oil portion of the grain, the remaining lipid in the grain was assumed to be replaced by a 97.5:2.5 (w/w) carbohydrate:lipid mixture. The results are termed oil-corrected biomass and oil-corrected grain yield in this paper. Although sunflower grain has a much higher proportion of protein than the remaining crop organs, no equivalent correction for protein synthesis costs was made, because most of the protein in the grain is derived from N taken up by the crop before the grain begins to grow rapidly (Hall et al., 1995). Estimates of total aerial biomass at A, total oil-corrected biomass at PM, and total oil-corrected biomass increase between A and PM were derived from bilinear functions (using the conditional model described in Section 2.2) fitted to total and grain oil-corrected biomass against DAA over the period from pre-A to post-PM (r^2 for functions fitted to total oil-corrected biomass vs. DAA, mean: 0.92, range: 0.65–0.99; r^2 for functions fitted to oil-corrected grain weight vs. DAA, mean: 0.93, range: 0.87–0.98).

2.4. Oil-corrected grain yield

Yield and yield determinants at physiological maturity were determined by hand harvesting of total aboveground biomass of 3.99 m^2 (one central row, discarding the border plants at both ends of the row). Grain oil concentration was determined on 10 g oven-dried achene samples by nuclear magnetic resonance (Granlund and Zimmerman, 1975). All grain (achene, includes kernel and husk) yield data is presented at 110 g kg^{-1} moisture.

2.5. LAI dynamics, radiation interception and radiation-use efficiency

Leaf area index dynamics were followed using measurements of maximum leaf width to determine leaf area of individual leaves (Pereyra et al., 1982) on 3 randomly selected plants per plot at weekly intervals from pre-A to past-PM, discounting the area of senesced leaves (a leaf was considered senescent when half or more of its area had yellowed). Leaf area index for each measurement date was calculated as the product of plot average leaf area per plant and crop population density. LAI dynamics were described by fitting 4th-order polynomials to LAI estimates as a function of time from pre-A. Values of r^2 for the fitted functions ranged from 0.967 to 0.999, with an overall mean of 0.990.

Fractional daily radiation interception (Qd) was estimated from daily LAI values derived from the above-described polynomials according to Orgaz et al. (1992). The usefulness of the combination of the Pereyra et al. (1982) and Orgaz et al. (1992) functions to estimate Qd has been previously verified by de la Vega and Hall (2002a). Daily intercepted radiation per plot was estimated using daily estimates of incident radiation and Qd, and used to construct the cumulative (0 = A) intercepted radiation values for the period A–PM. Mean A–PM radiation-use efficiency (RUE, g MJ^{-1} m^{-2}) of each replicate was estimated as the quotient between the total oil-corrected biomass increase (calculated as described in Section 2.3) and the cumulative intercepted radiation during this phase.

2.6. Rates of leaf senescence

SigmaPlot (SPSS Science, 2000) was used to fit piecewise bi-linear regressions to the LAI/time relationship for each experimental replicate (over the window from A minus 2–5 days to PM plus 7–10 days) to estimate the rates of leaf senescence. A conditional model was used with a first (slow) stage of LAI loss where:

$$\text{LAI} = a - b\,\text{DAA}$$

for DAA < C, and a second (fast) stage of LAI loss where DAA > C in which:

$$\text{LAI} = a + b\,C + d(\text{DDA} - \text{C})$$

where a and b are the intercept and the slope, respectively, of the linear regression corresponding to the first stage, DAA is days after full anthesis (R5.5 on the Schneiter and Miller (1981) scale), the constant C is the breakpoint (in DAA) of the function indicating the separation between the two stages (slow, fast) of leaf senescence, and d is the slope of the linear regression corresponding to the second stage. These bilinear plots were used to describe leaf senescence (see example, Fig. 2) instead of the polynomials used to estimate intercepted radiation because they provided a simple and effective means of establishing comparisons, between treatments, of the rates of the slow and fast phases of this process. In initial tests of this approximation, the breakpoint C was optimised, for each treatment replicate, by the SigmaPlot algorithm. Because the values of C were reasonably similar, but not invariable, within each experiment (range +/−4.4 days), and in order to improve comparability between treatments, a mean value of *C* was estimated for each experiment using optimised values, and the bilinear plots refitted using this mean as the fixed value of *C*. Range of r^2 values for these fitted bilinear plots with fixed *C* values was 0.84–0.99, with a mean of 0.98. The ratio of the unrestricted (i.e., replicate-specific) and restricted (i.e., a common value of *C* for each experiment) models' sums of error mean squares (i.e., 42 error MS values per model) was used to test the significance of restricting the *C* value within year, finding evidence of no significant differences between the two models ($F = 0.0107$, df1 = 202, df2 = 163, $P(>F) = 0.4736$).

2.7. Specific leaf N dynamics

Dynamics of specific leaf N (SLN, $\mathrm{g\,N\,m^{-2}}$) were followed during grain filling in the three experiments. At each sampling in Exp. 3, four randomly chosen plants from each plot were used, after excluding any plants that had been previously sampled. Samples (0.05-m diameter discs) were punched from the central portion of one side of the lamina (to avoid including the main leaf vein) of the 4th, 7th, 10th and 13th leaves from the capitulum (strata 1 to 4, respectively; leaf number per plant ranged from 30 to 38). Leaf discs were dried (48 h at 60 °C), and subsequently weighed and milled using a Cyclotec sample mill (Tecator AB, Hoganas, Sweden), and their N content determined by the micro Kjeldahl technique using a Se catalyst and a Tecator Kjeltec Autosystem IV (Tecator AB, Hoganas, Sweden). Less intensive sampling for SLN was conducted at weekly intervals in Exps. 1 and 2, using discs extracted from the 4th and the 6th leaf from the capitulum. These discs were bulked and their N-content determined as described for Exp. 3.

2.8. Resistance to stalk breakage

Resistance to stalk breakage was measured in Exp. 2 after PM and before commercial harvest, on ten consecutive plants in a row of each plot contiguous to the row devoted to measure grain yield, (i.e., plants were in perfect competition). The force (kg) necessary to break the stalk was obtained using an electronic balance (Model HEC, Balanzas Electrónicas Torres, Venado Tuerto), attached to the stalk at an approximate height of 1.5 m by a string. Force applied to the balance was increased gradually in a step-wise fashion while maintaining the string parallel to the ground. Maximum reading registered by the balance prior to stalk breakage was taken as the force needed to produce stem lodging. In addition to these measurements, performed on plants of Paraíso 20 and V82222 included in Exp. 2, similar measurements were performed on plants from nearby plots of V70597 grown (in a separate experiment) at the same density and planted on the same date as the plots of Exp. 2.

2.9. Statistical analyses

A mixed linear model was fitted for all measured and estimated attributes using the *lme* function of the *nlme* package of *R* (R Development Core Team, 2010). Genotype, plant density and their interaction were the fixed terms, while replicates within trials the random effects of the model. Residual non-homogeneous variances due to genotypes observed for some attributes were accommodated using the *varIdent* variance function. Multiple comparisons between means were performed using a procedure equivalent to L.S.D.-Fisher test. The analysis was performed through a user-friendly interface to the *nlme* package implemented in the InfoStat statistical software (Di Rienzo et al., 2010).

Complementary principal component and cluster analyses were applied to the attribute-standardised two-way (6 hybrids × 4 attributes) array of means of Exp. 3 to test the separation between the categories of NSG and SG using InfoStat (Di Rienzo et al., 2010). The attributes used in this analysis, selected on the basis of their likely value as indicators of functional stay-green, were: (i) $\mathrm{LAI_{PM}}$ as a % of $\mathrm{LAI_A}$; (ii) rate of leaf senescence during the second (rapid rate) phase (see above); (iii) A–PM increment in total oil-corrected biomass; and (iv) $\mathrm{RUE_{A-PM}}$. The matrix was centred by removal of attribute grand mean and normalised by division of the remainder by the within-attribute standard deviation (Fox and Rossielle, 1982). This standardisation is necessary since all attributes are recorded in different scales. For classification, a hierarchical agglomerative clustering method (Williams, 1976) with incremental sum of squares (Ward, 1963) as the fusion criterion was utilised. The squared Euclidean distance was used as the dissimilarity measure for Ward's method. A dendrogram was constructed on the basis of fusion level to group genotypes according to their relative responses for the four attributes. The principal components of the squared Euclidean distance matrices of hybrids × attributes were estimated using a singular value decomposition procedure and a one-dimensional plot of the first principal component was constructed from this analysis (Gabriel, 1971) for interpretation (Kempton, 1984).

To test the effect of genotypic category revealed by cluster analysis (i.e., SG and NSG) on the various measured attributes across all three experiments required accommodating the imbalance between datasets across experiments. To do this, data for all measured and estimated attributes were analysed as mixed models with separate residual terms for the different trials (van Eeuwijk et al., 2001; Smith et al., 2005). A compound symmetry model was employed in which genotypic group was assumed as fixed effect and the effects of hybrid nested within genotypic group, trial, replicate nested within trial and hybrid × trial interaction were assumed as random. Restricted maximum likelihood (Patterson and Thompson, 1975) using the sparse Average Information algorithm (Gilmour et al., 1995) was used to estimate the best linear unbiased estimates (BLUEs; means corrected by the imbalance of the dataset) of the genotypic groups, as implemented in GenStat 11.1 (2008).

3. Results

3.1. Growth conditions

Table 1 summarizes the seasonal patterns shown by the environmental variables registered for the three experiments. Accumulated rainfall during the pre-plant bare fallow [April–September interval] was 533, 421 and 199 mm for Exps. 1, 2 and 3, respectively, which was higher than average for the three experiments. In Exps. 1 and 2, rainfall during fallow exceeded the 340 mm plant available soil water store expected for a 3-m deep typic Hapludoll in the area of Venado Tuerto (Martín Silva Rossi, pers. comm.). Consequently, estimated seasonal soil water availability (i.e., the sum of in-season rainfall plus estimated soil water store at planting) should have been of the order of 827, 620 and 448 mm for Exps. 1, 2 and 3, respectively. Exp. 1 was exposed to higher-than-average rainfall during the crop cycle and higher-than-average minimum temperature during grain filling. Maximum temperatures were slightly lower than average in the three experiments for all crop phases. Plots were inspected and/or sampled every two-three days during the whole of grain-filling and up to a fortnight later, and no evidence was ever found of even quite mild water stress (e.g., leaf wilting in the early afternoon).

3.2. Categories of hybrids based on variables likely to reflect the stay-green syndrome

We choose to commence the presentation of our findings with the results of the principal component and cluster analyses applied

Table 1
Mean environmental data for three crop developmental phases in each trial. Anthesis (A) and physiological maturity (PM) dates used for defining the mean crop stages represent the average for all hybrids at each trial. Crop phase A represents the 15-d interval centred on the mean date of full anthesis. Crop interval S–A is the time elapsed between sowing and 7 days before the mean date of full anthesis; A–PM is the interval between 7 days after full anthesis and the mean date of PM (i.e., end of grain filling phase).

Environmental factor		Crop developmental phase		
		S–A	A	A–PM
Photoperiod (h)	*Exp. 1*	14.7	15.3	15.0
	Exp. 2	14.8	15.3	15.0
	Exp. 3	14.5	15.4	15.2
Maximum temperature (°C)	*Exp. 1*	24.6	30.2	29.0
	Exp. 2	25.9	29.6	29.2
	Exp. 3	26.8	28.7	29.3
Minimum temperature (°C)	*Exp. 1*	14.5	18.8	18.9
	Exp. 2	15.1	18.0	16.6
	Exp. 3	13.2	16.1	16.7
Daily incident radiation ($MJ\,m^{-2}$)	*Exp. 1*	18.5	22.8	22.3
	Exp. 2	20.7	20.1	22.9
	Exp. 3	20.9	22.4	25.3
Total growing season rainfall (mm)	*Exp. 1*	352.5	37.5	97.0
	Exp. 2	128.0	83.0	69.0
	Exp. 3	125.0	82.5	40.0

to the values for four attributes, selected on the basis of their likely value as indicators of the functional SG syndrome, measured in Exp. 3, in which the full set of six hybrids were grown together. The analysis clearly separated the six hybrids into three categories (Fig. 1), and partially coincided with our initial classification of the hybrids in putative SG and NSG types on the basis of visual observations of canopy leaf senescence patterns. Although hybrid V91705 could be classified as "fast dry down" according to its relative values for the attributes analysed (Fig. 1), the classification exercise (see Fig. 1, right) provides a formal basis for the SG and NSG labels used in the text of the subsequent subsections of Section 3. In the immediately following subsections of Section 3, we present a detailed analysis of the four attributes used in the principal component and cluster analyses, covering all three experiments.

3.3. LAI dynamics

There was a slight tendency for LAI at anthesis to be lower for the two SG hybrids than for the NSG hybrids, but these differences were only significant for some of the SG vs. NSG comparisons in Exps. 1 and 3, and were not significant in the REML combined analysis for the three experiments (Table 2). By contrast, LAI_{PM} was lower ($P = 0.065$) in the NSG hybrids as shown by the combined REML analysis for the pooled experiments, and was significantly lower in some of the SG vs. NSG contrasts in all three experiments (Table 2). When LAI_{PM} was expressed as a percentage of LAI_A (in order to account for possible differences between hybrids in LAI_A), the SG vs. NSG differences proved significant in the combined REML analysis and in all except one comparison in Exps. 1, 2 and 3 (Table 2). In Exps. 1 and 2, crop population density effects and crop population density by genotype interactions were not significant for these three variables.

As illustrated in Fig. 2, two stages could be distinguished in canopy senescence. In the first, which ran from just prior to anthesis to about 23 days after anthesis, rates of loss of canopy LAI were slow; in the second stage they were much faster. Double negative bilinear plots, fitted to data for each replicate using an experiment mean value (i.e., 28.3, 20.5 and 21.7 days after anthesis for Exps. 1, 2 and 3, respectively) for the breakpoint between the two stages, provided a very robust description of the process (r^2 = mean: 0.98, range: 0.84–0.99) and allowed for direct comparisons between treatments of rates of senescence in each of the two stages. We have no satisfactory explanation for the longer duration of the first phase in Exp. 1, although we note that the canopies in this experiment developed under lower radiation and slightly cooler temperatures than those of Exps. 2 and 3 (Table 1).

The combined REML analysis for rate of canopy senescence during the first stage (i.e., from approximately anthesis to the breakpoint between stages, cf. Fig. 2(a)) showed no significant effect for SG vs. NSG contrasts, and hybrid group effects within each experiment showed no consistent pattern (Table 3). By contrast, rates of canopy senescence in the second (fast phase) were always significantly smaller in the putative SG hybrids, both in individual experiments and in the combined REML analysis (Table 3). In Exp. 3 and among the NSG hybrids, V91705 had a particularly high rate of second-phase senescence (Table 3, Fig. 1).

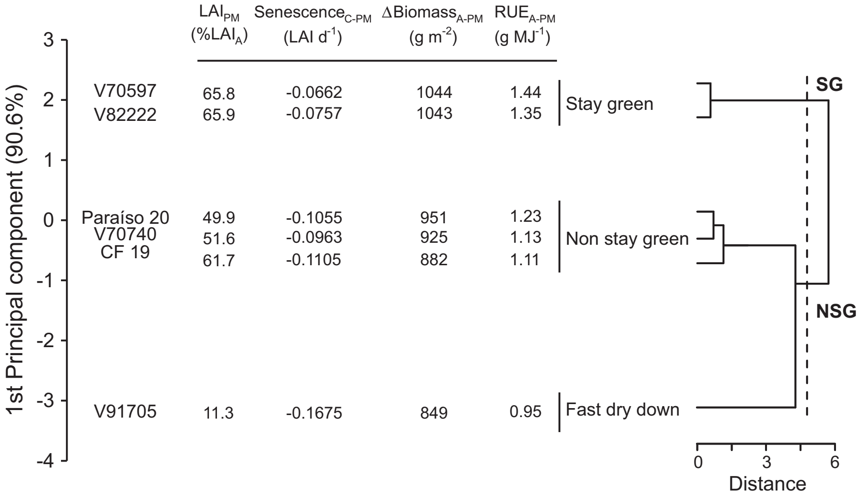

Fig. 1. First principal component (left side) and dendrogram (right side) showing relationships between 6 sunflower hybrids based on principal component and cluster analyses, respectively, of an attribute-standardised two-way (6 hybrids × 4 attributes) array of means from Exp. 3. Attributes used to construct the array of means (included in the figure to facilitate interpretation) were: (i) LAI at physiological maturity (PM) as a % of LAI at anthesis (A); (ii) rate of leaf senescence during the second (rapid rate) phase; (iii) A to PM increment in total oil-corrected biomass; and (iv) RUE for the A–PM interval. Data from Exp. 3.

Table 2
Mean ($n = 3$) values (individual experiments) and best linear unbiased estimates (REML combined analysis) and standard errors for leaf area index (LAI) at anthesis (A), at physiological maturity (PM) and LAI_{PM} as a % of LAI_A in Exps. 1, 2 and 3. Values at A and PM obtained from 4th order polynomials covering approximately the A – 5 days to PM + 15 days window of time. Results of analysis of variance are shown for each experiment separately, together with the REML analysis for the set of three experiments. For Exps. 1 and 2, the probability (P) of factor effects (δ, crop population density and G, genotype) and their interaction are shown. Means followed by a different letter are significantly ($P = 0.05$) different from other means within the same experiment.

Experiment/hybrid	LAI_A	LAI_{PM}	LAI_{PM} (% LAI_A)
Experiment 1 (2000/01)			
Paraíso 20 (δ_1)	4.93 ± 0.30 ab	1.49 ± 0.33b	29.9 ± 7.0b
V70597 (δ_1)	4.01 ± 0.30b	2.73 ± 0.33a	67.9 ± 7.0a
Paraíso 20 (δ_2)	5.05 ± 0.30a	1.94 ± 0.33ab	39.2 ± 7.0b
V70597 (δ_2)	3.92 ± 0.30b	2.19 ± 0.33ab	56.1 ± 7.0a
G	0.0130	0.0495	0.0013
P δ	0.9542	0.8883	0.8025
G × δ	0.7342	0.1586	0.0717
Experiment 2 (2001/02)			
Paraíso 20 (δ_1)	5.29 ± 0.46a	1.22 ± 0.30b	23.0 ± 6.8b
V82222 (δ_1)	5.69 ± 0.46a	3.55 ± 0.30a	63.4 ± 6.8a
Paraíso 20 (δ_2)	5.48 ± 0.46a	1.00 ± 0.30b	19.5 ± 6.8b
V82222 (δ_2)	5.33 ± 0.46a	3.41 ± 0.30a	64.9 ± 6.8a
G	0.6805	0.0002	<0.0001
P δ	0.7674	0.5697	0.7966
G × δ	0.3559	0.8987	0.5423
Experiment 3 (2003/04)			
CF 19	4.13 ± 0.26a	2.54 ± 0.20a	61.7 ± 4.5ab
Paraíso 20	3.81 ± 0.26ab	1.90 ± 0.20b	49.9 ± 4.5b
V70740	4.03 ± 0.26ab	2.08 ± 0.20ab	51.6 ± 4.5b
V91705	3.27 ± 0.26b	0.42 ± 0.20c	11.3 ± 4.5c
V82222	3.57 ± 0.26ab	2.34 ± 0.20ab	65.9 ± 4.5a
V70597	3.24 ± 0.26b	2.13 ± 0.20ab	65.8 ± 4.5a
P	0.0054	0.0002	0.0001
REML combined analysis			
Non stay green	4.638	1.54	35.2
Stay green	4.385	2.49	61.6
S.E.D.	0.27	0.45	9.5
P	0.381	0.065	0.026

δ: Crop population density, with $\delta_1 = 4.51$ plants m^{-2} and $\delta_2 = 6.90$ plants m^{-2}; S.E.D.: standard error of the difference between means.

3.4. Post-anthesis biomass increase

Total aerial biomass at anthesis was significantly greater in the NSG hybrids (REML combined analysis, Table 4), an effect which also proved significant in many, but not all, SG vs. NSG comparisons within each of Exps. 1, 2 and 3 (Table 4). At PM, these differences had been erased (Table 4). The biomass increase between A and PM was significantly greater for the SG hybrids when the three experiments were pooled (REML combined analysis, Table 4), with data for individual experiments showing the same trend (Table 4).

3.5. Post-anthesis cumulative intercepted radiation and radiation use efficiency

Cumulated intercepted radiation between anthesis and physiological maturity did not differ significantly between hybrid groups (REML combined analysis for the three experiments), and any differences between categories for this variable in individual experiments favoured the NSG hybrids (Table 5). Mean post-anthesis RUE for SG hybrids was greater ($P = 0.052$), across the three experiments, than the NSG hybrids (Table 5), and where significant differences between SG and NSG hybrids were found within an experiment (e.g., Exps. 1 and 3), SG hybrids exhibited the higher RUE values (Table 5).

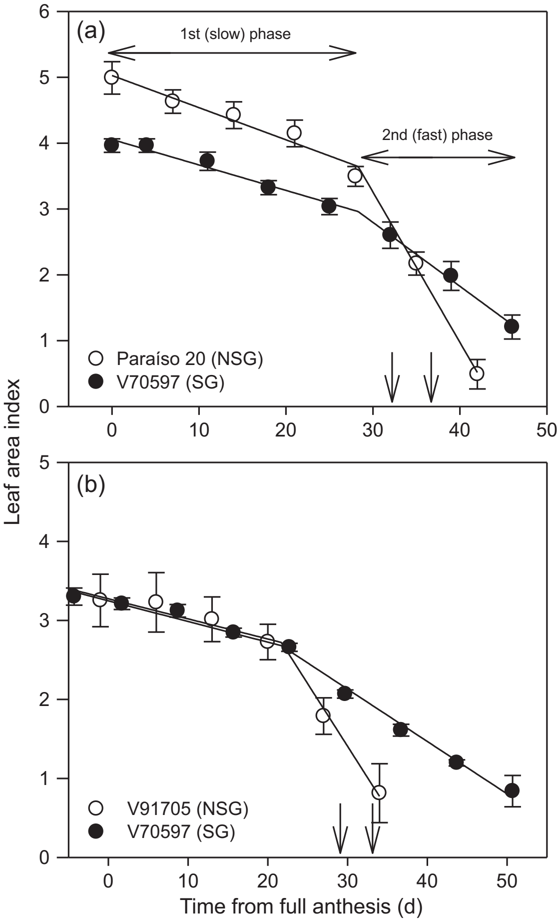

Fig. 2. Two examples of LAI dynamics during grain filling for pairs of hybrids exhibiting contrasting patterns of canopy senescence taken from Exps. 1 (Fig. 1(a)) and 3 (Fig. 1(b)). Horizontal double-ended arrows in Fig. 1(a) show stages (slow and fast) of LAI loss, vertical arrows indicate PM for each hybrid (V70597 on the left side in both figures). Data points are means of three replicates, error bars are SEs. Lines for stages 1 and 2 have slopes equal to treatment means and are shown for illustration purposes only. This figure was constructed by fitting double negative bilinear functions to each replicate separately and then obtaining a mean value for the regression variables that define the lines shown in the figures.

3.6. Specific leaf N dynamics

Specific leaf N in all four leaf-strata of the 6 hybrids followed in Exp. 3 exhibited a gently falling trend over the first 20 to 22 days after flowering, followed by a sharper fall to a new, rather stable, level that commenced about 33 days after flowering (Fig. 3). Between-hybrid comparisons of pooled SLN values for Strata 1 + 2 and Strata 3 + 4 for the first phase (i.e., prior to 22 DAA, see Fig. 3) of canopy senescence showed that Paraíso 20 has significantly lower ($P < 0.02$) SLN than the remaining hybrids. Equivalent comparisons for individual strata showed the same effect ($P < 0.02$ for Strata 1, 3 and 4) (data not shown). These between-hybrid differences in SLN disappeared in the second phase of canopy senescence (i.e., between 28 and 40 DAA, see Fig. 3), (statistical analyses not shown). The results of less intensive sampling for SLN in Exps. 1 and 2 were consistent with this observation: in Exp. 1 levels of SLN in Paraíso 20 were lower than those of V70597 ($P = 0.084$, data not shown); in Exp. 2 levels of SLN in Paraíso 20 were significantly lower than those of V82222 ($P = 0.0029$, data not shown). A limited number of readings using a chlorophyll meter (SPAD-502, Minolta, Plainfield, IL) during Exp. 1 were also consistent with the SLN measurements in showing that Paraíso 20 had lower levels of chlorophyll than V70597 over the period 15 to 35 DAA (data not shown).

Table 3

Mean ($n = 3$) values (individual experiments) and best linear unbiased estimates (REML combined analysis) and standard errors for rates of canopy senescence during the slow and fast phases of canopy loss. Results of analysis of variance are shown for each experiment separately, together with the REML analysis for the combined set of three experiments. For Exps. 1 and 2, the probability (P) of factor effects (δ, crop population density and G, genotype) and their interaction are shown. Means followed by a different letter are significantly ($P = 0.05$) different from other means within the same experiment.

Experiment/hybrid	Rate of leaf senescence$_{A–C}$ (LAI d^{-1})	Rate of leaf senescence$_{C–PM}$ (LAI d^{-1})
Experiment 1 (2000/01)		
Paraíso 20 (δ_1)	−0.0547 ± 0.0072b	−0.2144 ± 0.0210b
V70597 (δ_1)	−0.0322 ± 0.0072a	−0.0851 ± 0.0210a
Paraíso 20 (δ_2)	−0.0428 ± 0.0072ab	−0.2434 ± 0.0210b
V70597 (δ_2)	−0.0484 ± 0.0072b	−0.0937 ± 0.0210a
P G	0.0542	0.0001
P δ	0.5635	0.2928
P G × δ	0.0074	0.5549
Experiment 2 (2001/02)		
Paraíso 20 (δ_1)	−0.0645 ± 0.0150a	−0.2544 ± 0.0383b
V82222 (δ_1)	−0.0417 ± 0.0150a	−0.0820 ± 0.0383a
Paraíso 20 (δ_2)	−0.0577 ± 0.0150a	−0.2900 ± 0.0383b
V82222 (δ_2)	−0.0460 ± 0.0150a	−0.0751 ± 0.0383a
P G	0.1493	0.0001
P δ	0.9074	0.5216
P G × δ	0.6152	0.3525
Experiment 3 (2003/04)		
CF 19	−0.0206 ± 0.0026b	−0.1105 ± 0.0067b
Paraíso 20	−0.0309 ± 0.0059ab	−0.1055 ± 0.0087bc
V70740	−0.0366 ± 0.0019a	−0.0963 ± 0.0065c
V91705	−0.0246 ± 0.0062ab	−0.1675 ± 0.0220a
V82222	−0.0223 ± 0.0049b	−0.0757 ± 0.0105d
V70597	−0.0260 ± 0.0057ab	−0.0662 ± 0.0063d
P	0.0102	<0.0001
REML combined analysis		
Non stay green	−0.0417	−0.1867
Stay green	−0.0394	−0.0932
S.E.D.	0.0044	0.0273
P	0.612	0.014

δ: Crop population density, with δ_1 = 4.51 plants m^{-2} and δ_2 = 6.90 plants m^{-2}; S.E.D.: standard error of the difference between means.

Table 4

Mean ($n = 3$) values (individual experiments) and best linear unbiased estimates (REML combined analysis) and standard errors for aerial biomass at anthesis (A) and physiological maturity (B [corrected for oil synthesis costs]) and biomass increment (Δ) between A and PM. Results of analysis of variance are shown for each experiment separately, together with the REML analysis for the combined set of three experiments. For Exps. 1 and 2, the probability (P) of factor effects (δ, crop population density and G, genotype) and their interaction are shown. Means followed by a different letter are significantly ($P = 0.05$) different from other means within the same experiment.

Experiment/hybrid	Total aerial biomass$_A$ (g m^{-2})	Total oil-corrected biomass$_{PM}$ (g m^{-2})	Δ Total oil-corrected biomass$_{A–PM}$ (g m^{-2})
Experiment 1 (2000/01)			
Paraíso 20 (δ_1)	802 ± 62b	1934 ± 85ab	1133 ± 121a
V70597 (δ_1)	597 ± 62b	2050 ± 85ab	1453 ± 121a
Paraíso 20 (δ_2)	1042 ± 62a	2158 ± 85ab	1116 ± 121a
V70597 (δ_2)	757 ± 62b	2201 ± 85a	1444 ± 121a
P G	0.0076	0.2998	0.0361
P δ	0.0181	0.0366	0.9171
P G × δ	0.5437	0.6244	0.9752
Experiment 2 (2001/02)			
Paraíso 20 (δ_1)	1019 ± 58c	2407 ± 178a	1388 ± 193a
V82222 (δ_1)	1135 ± 58bc	2247 ± 178a	1112 ± 193a
Paraíso 20 (δ_2)	1459 ± 58a	2661 ± 178a	1201 ± 193a
V82222 (δ_2)	1229 ± 58b	2735 ± 178a	1506 ± 193a
P G	0.3649	0.8199	0.9422
P δ	0.0037	0.0823	0.6099
P G × δ	0.0245	0.5360	0.1828
Experiment 3 (2003/04)			
CF 19	807 ± 17ab	1689 ± 94a	882 ± 100a
Paraíso 20	873 ± 31a	1824 ± 94a	951 ± 100a
V70740	838 ± 26ab	1763 ± 94a	925 ± 100a
V91705	838 ± 30ab	1687 ± 94a	849 ± 100a
V82222	707 ± 11c	1750 ± 94a	1043 ± 100a
V70597	716 ± 50bc	1760 ± 94a	1044 ± 100a
P	0.0006	0.8987	0.6555
REML combined analysis			
Non stay green	984	2088	1091
Stay green	879	2126	1249
S.E.D.	41	52	60
P	0.043	0.477	0.014

δ: Crop population density, with δ_1 = 4.51 plants m^{-2} and δ_2 = 6.90 plants m^{-2}; S.E.D.: standard error of the difference between means.

3.7. *Oil-corrected grain yield and its determinants*

The REML combined analysis for the pooled results of the three experiments showed no significant differences in oil-corrected grain yield between SG and NSG hybrids (Table 6). However, significant differences for this trait were observed within individual years. In Exps. 1 and 2, the effect of plant density was significant; the high plant population being the one associated with higher yields. Genotypic effect was significant in Exps. 2 and 3; the SG hybrid V82222 showing higher oil-corrected yield than the NSG hybrid Paraíso 20 in Exp. 2 and the SG hybrid V70597 showing lower yield than the NSG hybrids Paraíso 20 and CF 19 in Exp. 3. If the rather variable oil-corrected yield data shown by V91705 in Exp 3 are removed from the analysis, the SG hybrid V82222 also showed significantly lower yield than Paraíso 20 and CF 19 in this trial ($P = 0.0163$; data not shown). The genotype × plant density interaction effect was significant in Exp. 2 because the NSG hybrid Paraíso 20 showed a relatively higher improvement in oil-corrected yield at high plant density than the SG hybrid V82222 (Table 6). Analysis of pooled data for time to anthesis from the three experiments also suggested that differences in time to anthesis between SG and NSG were not significant, but examination of ANOVA results for the individual experiments indicate that, with one exception (contrasts with CF 19 in Exp. 3), time to anthesis in SG hybrids was 2–6 days shorter than that of the NSG hybrids, and that this difference was significant (Table 6). Duration of grain filling tended ($P = 0.052$) to be shorter in SG hybrids than in NSG ones, significantly so in some of the comparisons in Exps. 1 and 3 (Table 6).

3.8. *Resistance to stalk breakage*

Stay-green hybrids showed a significantly higher resistance to stalk breakage than NSG hybrid Paraíso 20 across both normal and high plant population densities (Table 7). A significant non-crossover genotype × plant density interaction was also observed, mainly due to a relatively higher reduction in the stalk breakage resistance of V70597 at high crop population density (Table 7).

4. Discussion

Because leaf senescence and attributes such as RUE are susceptible to water stress and crop seasonal water balance can be affected by genotype-dependent LAI dynamics, it is important to consider the likelihood that water stress might have affected our results. Modelling (Grassini et al., 2009) and direct measurement (Dardanelli et al., 2003) have shown that seasonal evapotranspiration of a sunflower crop growing without water stress under similar climatic and soil conditions to those of Venado Tuerto does not exceed the order of 630 mm, a value lower or similar to that of our estimate of seasonal water availability in Exps. 1 and 2. A value of 447.5 mm for seasonal water availability in Exp. 3 falls below this

Table 5
Mean ($n = 3$) values (individual experiments) and best linear unbiased estimates (REML combined analysis) and standard errors for post-anthesis cumulative intercepted radiation and radiation use efficiency. Results of analysis of variance are shown for each experiment separately, together with the REML analysis for the combined set of three experiments. For Exps. 1 and 2, the probability (P) of factor effects (δ, crop population density and G, genotype) and their interaction are shown. Means followed by a different letter are significantly ($P = 0.05$) different from other means within the same experiment.

Experiment/hybrid	Accumulated intercepted radiation$_{\text{A–PM}}$ (MJ m^{-2})	Radiation-use efficiency$_{\text{A–PM}}$ (g MJ^{-1})
Experiment 1 (2000/01)		
Paraíso 20 (δ_1)	803 ± 25.2ab	1.41 ± 0.18b
V70597 (δ_1)	758 ± 25.2ab	1.91 ± 0.18ab
Paraíso 20 (δ_2)	829 ± 25.2a	1.34 ± 0.18b
V70597 (δ_2)	727 ± 25.2b	2.28 ± 0.18a
G	0.0269	0.0073
P δ	0.9360	0.4486
G × δ	0.2962	0.2801
Experiment 2 (2001/02)		
Paraíso 20 (δ_1)	712 ± 30.1a	1.96 ± 0.28a
V82222 (δ_1)	757 ± 30.1a	1.48 ± 0.28a
Paraíso 20 (δ_2)	728 ± 30.1a	1.67 ± 0.28a
V82222 (δ_2)	702 ± 30.1a	2.19 ± 0.28a
G	0.7053	0.9406
P δ	0.4578	0.4729
G × δ	0.1978	0.1204
Experiment 3 (2003/04)		
CF 19	808 ± 38.1a	1.11 ± 0.13b
Paraíso 20	786 ± 38.1ab	1.23 ± 0.11ab
V70740	811 ± 38.1a	1.13 ± 0.15ab
V91705	776 ± 38.1ab	0.95 ± 0.14b
V82222	771 ± 38.1ab	1.35 ± 0.13ab
V70597	701 ± 38.1b	1.44 ± 0.05a
P	0.2729	0.0496
REML combined analysis		
Non stay green	777	1.42
Stay green	746	1.68
S.E.D.	23	0.11
P	0.246	0.052

δ: Crop population density, with $\delta_1 = 4.51$ plants m^{-2} and $\delta_2 = 6.90$ plants m^{-2}; S.E.D.: standard error of the difference between means.

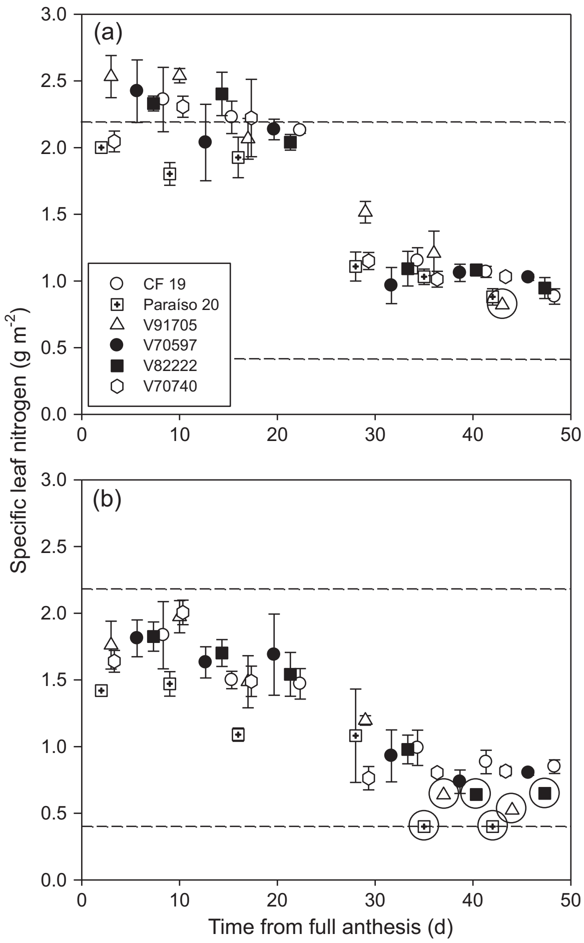

Fig. 3. Specific leaf N dynamics for leaf strata 1 (a) and 4 (b), i.e., 4th and 13th leaves, respectively, from the capitulum for the six hybrids used in Exp. 3. Data points are mean values ($n = 3$) and error bars are SEs. Horizontal dashed lines show estimates of critical (upper) and minimum (lower) values of SLN derived from reports of single-leaf $P_{\max}$ responses to SLN by Connor et al. (1993), Trápani and Hall (1996) and Bange et al. (1997). Circled symbols are estimates obtained assigning the minimum value to senesced leaves on sample plants and are shown without SEs.

threshold, but our estimate is only based pre-plant bare fallow rainfall (i.e., it excludes any estimate of residual available soil water left in the profile by the crop that preceded the fallow). Average grain yields for the three experiments were high (3755, 4698 and 4373 kg ha^{-1} in Exps. 1, 2 and 3, respectively). Using the boundary line function for the grain yield/seasonal available soil water relationship developed by Grassini et al. (2009) (i.e., 8 kg ha^{-1} mm^{-1}, with an x-intercept of 75 mm) suggests that seasonal evapotranspiration in Exp. 3 would have been in the order of 622 mm (i.e., there must have been some residual moisture in the profile at the start of the fallow). These analyses, together with the lack of any symptoms of wilting in the plots of all three experiments, give us confidence that our crops grew essentially without water stress throughout the season.

Taken together, the results of the combined REML analysis of the pooled data for the three experiments showing significantly slower second phase rates of canopy senescence (Fig. 2, Table 3), significantly greater residual canopy LAI$_{\text{PM}}$ as a percentage of that present at anthesis (Table 2), significantly greater A–PM biomass increase (Table 4), and greater post-anthesis RUE (Table 5), strongly support the contention that the two putative SG sunflower hybrids used in these experiments exhibit functional stay green. The results of the cluster analysis (Fig. 1) and those of analysis of variance for individual experiments (Tables 2–5) serve to strengthen this interpretation. Our results constitute the first report of functional canopy stay green in a set of well adapted sunflower hybrids sown at their optimum sowing date (i.e., not differences in stay green evoked by exposure to extremely late sowing dates [de la Vega and Hall, 2002a]).

The main features of SLN dynamics across leaf strata (i.e., an initial value which falls from leaf 4 to leaf 13 and a simultaneous loss of N across all strata) were consistent with those reported by Sadras et al. (1993). Fig. 3 shows the critical and minimum limits for the $P_{\max}$ (i.e., photosynthetic rate at full sunlight, cf. Connor et al., 1993) response to SLN, derived from data on single-leaf photosynthesis published by Connor et al. (1993), Trápani and Hall (1996) and Bange et al. (1997). Our data (Fig. 3(a)) suggest that, with the exception of Paraíso 20 (see below), SLN values in the uppermost leaf stratum were close to the critical limit until around 20–22 days after anthesis and only exceptionally fell to the minimum value in samples taken well after physiological maturity. By contrast, initial values of SLN in stratum 4 leaves (Fig. 3(b)) were lower than the critical limit at the start of sampling and fell to values close to the minimum limit at the close of sampling. SLN dynamics in Stratum 2 was close to those of Stratum 1 and those of Stratum 3 close to those of Stratum 4 (data not shown). Interestingly, the switch from first to second phase of LAI loss (Fig. 2(b)) and the acceleration of the rate of loss of N from the leaves (Fig. 3) appear to be synchronous. This synchronicity may reflect the fact that N draw-down in all leaves of the crop occurs simultaneously, albeit

Table 6
Mean ($n = 3$) values (individual experiments) and best linear unbiased estimates (REML combined analysis) and standard errors for oil-corrected grain yield, time to full anthesis (R5.5 on the Schneiter and Miller (1981) scale), and duration of grain filling (in days after full anthesis). Results of analysis of variance are shown for each experiment separately, together with the REML analysis for the combined set of three experiments. For Exps. 1 and 2, the probability (P) of factor effects (δ, crop population density and G, genotype) and their interaction are shown. Means followed by a different letter are significantly ($P = 0.05$) different from other means within the same experiment.

Experiment/hybrid	Oil-corrected grain yield ($g\,m^{-2}$)	Time to full anthesis (d)	Duration of grain filling (d)
Experiment 1 (2000/01)			
Paraíso 20 (δ_1)	625 ± 29a	83 ± 0.17a	36.9 ± 1.46a
V70597 (δ_1)	616 ± 29a	77 ± 0.17b	33.5 ± 1.46a
Paraíso 20 (δ_2)	685 ± 29a	83 ± 0.17a	37.1 ± 1.46a
V70597 (δ_2)	689 ± 29a	77 ± 0.17b	32.4 ± 1.46a
P G	0.9307	<0.0001	0.0306
δ	0.0635	0.3559	0.7839
G × δ	0.8365	0.3559	0.6676
Experiment 2 (2001/02)			
Paraíso 20 (δ_1)	708 ± 32b	75 ± 0.24a	32.7 ± 1.31a
V82222 (δ_1)	861 ± 32a	73 ± 0.24b	33.6 ± 1.31a
Paraíso 20 (δ_2)	852 ± 32a	76 ± 0.24a	34.4 ± 1.31a
V82222 (δ_2)	894 ± 32a	73 ± 0.24b	31.8 ± 1.31a
P G	0.0071	0.0001	0.5194
δ	0.0111	0.4680	0.9239
G × δ	0.0623	0.0591	0.2106
Experiment 3 (2003/04)			
CF 19	858 ± 8a	77 ± 0.64c	33.2 ± 1.53abc
Paraíso 20	864 ± 36a	83 ± 0.64a	33.4 ± 1.53abc
V70740	812 ± 40ab	82 ± 0.64a	33.9 ± 1.53ab
V91705	744 ± 121ab	82 ± 0.64a	37.3 ± 1.53a
V82222	715 ± 61ab	78 ± 0.64bc	31.9 ± 1.53bc
V70597	697 ± 41b	79 ± 0.64b	29.3 ± 1.53c
P	0.0273	<0.0001	0.0518
REML combined analysis			
Non stay green	766	79	35.0
Stay green	745	77	32.3
S.E.D.	44	1.65	1.15
P	0.645	0.213	0.052

δ: Crop population density, with $\delta_1 = 4.51$ plants m^{-2} and $\delta_2 = 6.90$ plants m^{-2}; S.E.D.: standard error of the difference between means.

from an initial value which falls with accumulated LAI from the top of the canopy (Sadras et al., 1993, our Fig. 3). We did not follow SLN dynamics in leaves lower than the 13th position below the capitulum, but if all leaf-levels below the ones we sampled followed a pattern of SLN dynamics similar to those shown in Fig. 3, once N draw-down accelerated, leaves at the lowest levels would have quickly reached their minimum SLN values and have become senescent, thus reducing LAI.

The lower capacity for post-anthesis biomass increase in NSG hybrids could not be ascribed either to lower intercepted radiation (Table 5) nor shorter durations of grain filling (Table 6). Two candidate explanations for the overall equal or greater capacity of the SG hybrids to accumulate greater biomass in post-anthesis via increased RUE, in some cases in spite of a lower LAI_A (Table 2), are their SLN (see previous paragraph) and LAI (Villalobos et al., 1996; Soriano et al., 2004) dynamics. The SLN dynamics exhibited by Stratum 1 leaves of Paraíso 20 (Fig. 3(a)), with values in the first three weeks after anthesis that were below the critical SLN value for P_{max}, would certainly have contributed to lowering the RUE of this hybrid. Lower SLN values for Paraíso 20 in the remaining three leaf strata may also have contributed to this effect. We conclude that the lower (with respect to other hybrids) SLN values in Paraíso 20 in the three experiments played an important part in determining SG vs. NSG differences in contrasts involving this hybrid. It is appropriate, here, to note that insofar as the SLN/SG linkage is concerned, three of the four NSG hybrids used in these experiments appear to differ from the pattern found in sorghum and maize (Borrell and Hammer, 2000; He et al., 2005; van Oosterom et al., 2010b), in which NSG types have lower SLN. Insofar as the LAI dynamics are concerned, Villalobos et al. (1996) and Soriano et al. (2004) showed that sunflower RUE is a positive function of LAI in the 0.5–2.5 range, and we speculated that the greater canopy retention of the SG hybrids (Table 2) might have contributed to their higher (with respect to NSG hybrids of similar SLN) post-anthesis RUE. However, careful inspection of LAI dynamics in all possible pairwise (one SG, the other NSG) comparisons between hybrids within all experiment replicates between SG (V70957 and V82222) vs. NSG (CF19, V70740, V91705) hybrids showed that the LAI trajectory of the NSG hybrids fell below those of the SG hybrids very close to or just after physiological maturity of the SG hybrid (which, in each comparison, preceded that of the NSG hybrid, cf. Table 6 and Fig. 2), with the sole exception of the contrasts involving V91705, the NSG hybrid with the fastest rate of LAI senescence (Exp. 3, Fig. 2(b)). Leaf area index values for Paraíso 20 also fell below those of V82222 before PM of the latter in Exp. 2, and this could have contributed to the greater RUE of V82222 in the δ_2 treatment in that experiment. Thus, we are unable to explain the lower RUE values of NSG hybrids other than those of Paraíso 20 and V91705. It is possible that the SLN values of the SG hybrids may have held up a bit longer than the NSG hybrids in the transition between the early and late phases of the trajectory of this variable (Fig. 3), but this hypothesis cannot be tested with the data available.

A somewhat disappointing result of this research was the fact that the SG hybrids did not appear to be able to translate their capacity for post-anthesis biomass accumulation into grain yield. A possible contributing factor was the lower biomass at anthesis of the SG hybrids across years (Table 4), linked to shorter times to full anthesis (Table 6). Grain yield was strongly correlated with grain number across all three experiments ($y = 423 + 0.043x$, $P < 0.0001$; data not shown), and this variable was, in turn, associated with biomass at anthesis ($y = 2816 + 5.52x$; $P < 0.0001$; data not shown). Differences between years in terms of genotypic discrimination for oil-corrected yield support this notion, given that the SG hybrid V82222 showed a significantly higher yield than the NSG hybrid Paraíso 20 across plant densities in the only experiment in which no significant differences in biomass at anthesis were detected (i.e., Exp. 2, Tables 4 and 6). A second factor which may have militated against greater yield in the SG hybrids was their tendency to have shorter durations of grain filling (Table 6, Fig. 2), thus reducing their potential for radiation interception during post-anthesis. For SG to be an attribute of value for enhancing yield in non- or slightly stressed environments, it needs to be combined with sufficient

Table 7
Force required to produce stem breakage. Values are means (10 plants per replicate, 3 replicates per treatment), estimates of least significant differences (L.S.D.) taken from ANOVA. Data for hybrids included in Exp. 2 and additional plots of hybrid V70597 sown on the same date as Exp. 2.

Hybrid	Mean stalk breakage resistance (kg)		Source of variation	P	L.S.D. $\alpha = 0.10$
	4.51 plant m^{-2}	6.90 plant m^{-2}			
Paraíso 20	0.91	0.95	Genotype	0.001	0.54
V70597	3.32	1.65	Plant density	0.036	0.44
V82222	1.54	1.42	Genotype × plant density	0.030	0.76

time to anthesis and sufficient duration of grain-filling. Because N source/sink relationships have been shown to play an important part in determining stay-green in other species (e.g. van Oosterom et al., 2010a,b), there is a need to conduct whole crop N balance dynamics studies to deal with this issue. It is tempting to speculate that the tendency toward lower yields in the SG hybrids is associated with a lower demand for N by the grain resulting, in turn, in less leaf senescence. However, this is counteracted by a tendency toward lower LAI_A in the SG hybrids (Table 2) and the results of Exp. 2, which show that at similar values of LAI_A (Table 2) and yield (Table 6), V82222 showed a slower second-phase loss of LAI than Paraíso 20 (Table 3).

The SG hybrids required greater force to produce stem breakage (Table 7). Since an estimated 10% of the Argentine sunflower crop is lost to stem and root lodging every year (Bragachini et al., 2001), this result makes SG an interesting secondary trait in selection for higher and more stable yields in this crop species.

Given that our results show there is variability for functional SG in the sunflower germplasm, two outstanding issues that require further research are the relative contributions of LAI and SLN dynamics to post-anthesis biomass accumulation (see above) and the possible contribution of the SG attribute to yield stability in water-stressed environments. It is possible that a 3-D modelling exercise (e.g., Birch et al., 2003) of the post-anthesis sunflower canopy which incorporates both SLN and LAI dynamics could provide an answer to the first issue. Experimentation is needed to explore the second issue in order to establish whether the advantages for SG in water-stressed environments found in other species (e.g., Borrell et al., 2000; Clifton-Brown et al., 2002) also apply to sunflower.

In summary, our experiments and analyses have served to demonstrate that functional SG exists in sunflower, that post-anthesis SLN dynamics is a factor in some, but not all SG/NSG contrasts, that SG needs to be combined with other attributes (time to anthesis, duration of grain filling) in order to contribute to grain yield, and that SG improves resistance to stalk breakage.

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