



Carbon assimilation, leaf area dynamics, and grain yield in contemporary earlier- and later-senescing maize hybrids



Horacio A. Acciaresi^{a,d}, Eduardo A. Tambussi^{b,e}, Mariana Antonietta^{b,e}, María S. Zuluaga^a, Fernando H. Andrade^{c,e}, Juan J. Guiamet^{b,d,*}

^a Cátedra de Cerealicultura, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, cc 31, 1900 La Plata, Argentina

^b Instituto de Fisiología Vegetal (CONICET-UNLP), cc 327, 1900 La Plata, Argentina

^c Unidad Integrada INTA Balcarce-Universidad Nacional de Mar del Plata, cc 276, 7620 Balcarce, Argentina

^d CICBA, Argentina

^e CONICET, Argentina

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ABSTRACT

Maize breeding during the past 50 years has been associated with a delay of leaf senescence, but it is not clear whether this trait is likewise associated with higher grain yield in modern hybrids. Post-silking growth, leaf area dynamics, photosynthetic parameters and yield were compared in modern maize hybrids differing in canopy senescence rate. In the first two experiments, four hybrids were grown in the field at Balcarce, Argentina (37°45' S, 58°18' W). In spite of differences in chlorophyll retention and photosynthesis of the ear leaf, post-silking growth and grain yield were very similar in all four hybrids while kernel N concentration was lower in the later-senescing hybrids. In a third experiment, a later-senescing (NK870) and an earlier-senescing (DK682) hybrid were grown to analyze the potential photosynthetic contribution of delayed leaf senescence. Leaf area and chlorophyll content were larger in NK870, especially at the lower canopy level (0.75 m above the ground). However, hybrids did not differ for canopy light interception. Because photosynthetic photon flux density below 1 m above the ground was less than 10% of incident radiation and photosynthesis quantum yield did not change during senescence, the potential photosynthetic output of lower leaves below 1 m was very low. Lower leaves of NK870 had N concentrations higher than those needed to sustain photosynthesis at the light conditions below 1 m. Therefore, we show that delayed senescence does not necessarily improve post-silking C accumulation because: (i) canopy light interception is not reduced by senescence except at very late stages of grain filling; (ii) contrasting hybrids show more pronounced senescence differences at canopy levels receiving less than 10% of incident radiation; (iii) delayed senescing hybrids present lower kernel N concentrations while extra N is retained in leaves exposed to a light limiting micro-environment. Delayed senescence at lower canopy levels may be unproductive, at least under non-stressing conditions.

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1. Introduction

Maize grain yield increased significantly over the past 50 years. For example, the rate of yield increase in Ontario (Canada) approximated 1.5% yr⁻¹ between 1940 and 1990 (Tollenaar and Wu, 1999), whereas average maize yields doubled between 1969 and 2007 in Argentina (www.minagri.gob.ar). In part, this increase has been due to the release of improved hybrids. Genetic gains for yield equalled 13.2 g m⁻² yr⁻¹ between 1965 and 1997 in Argentina (Luque et al.,

2006). Genetic improvement of maize yield can be related to increased tolerance to various types of stress (e.g., low soil water and/or N availability, increased population density, competition from weeds) and also to increased post-silking dry matter accumulation (Tollenaar and Wu, 1999). To some extent, this has been associated with an improvement in resource capture (light, water, and nutrients). Prolonged canopy functioning through delayed leaf senescence is one of several traits that have contributed to the increased yield potential of the new hybrids (Tollenaar and Wu, 1999; Valentinuz and Tollenaar, 2004).

In Argentina, maize canopy senescence proceeds at a relatively slow rate (1.4 cm² per plant°C d⁻¹) until silking – 400°C d after silking, when rates of green leaf area loss climb to 5.5 cm² per plant°C d⁻¹ (Borrás et al., 2003). The second, faster phase of senescence coincides with the reproductive stage (Borrás et al.,

* Corresponding author at: Instituto de Fisiología Vegetal (CONICET-UNLP), cc 327, 1900, La Plata, Argentina. Tel.: +54 221 4236618; fax: +54 221 4233698.

E-mail addresses: jguamet@fcnym.unlp.edu.ar, jguamet@gmail.com (J.J. Guiamet).

2003). The onset and/or the rate of senescence varies depending on environmental conditions, year, population density, and source–sink relations. Increased population density and decreased source–sink ratios frequently accelerate senescence (Rajcan and Tollenaar, 1999b; Borrás et al., 2003; Valentinuz and Tollenaar, 2004; Antonietta et al., 2014). Compared to older genotypes, senescence occurs later or at slower rates in modern hybrids (e.g., Valentinuz and Tollenaar, 2004; Duvick, 2005), i.e., chlorophyll and photosynthetic activity persist longer after silking in modern hybrids (Ding et al., 2005). In maize and sorghum, delayed senescence is associated with increased C and N assimilation during the grain filling period (Ma and Dwyer, 1998; Rajcan and Tollenaar, 1999a,b; Borrell et al., 2000a,b). Later-senescing hybrids of maize exhibit increased source–sink ratios during grain filling, as implied from their smaller decrease of stover mass compared to older, earlier-senescing hybrids (Rajcan and Tollenaar, 1999a). This has led to the proposal that an increased source–sink ratio during grain filling results in increased supply of assimilates to the roots in these genotypes. Persistence of root activity might extend assimilation of N up to physiological maturity. In fact, several studies have shown that the percentage of grain N supplied by current N assimilation during grain filling is larger in delayed senescence than in normally senescing hybrids (Ma and Dwyer, 1998; Rajcan and Tollenaar, 1999b; He et al., 2004).

In most hybrids, loss of green leaf area starts at the base of the plant, and progresses upwards during reproductive growth (e.g., Sadras et al., 2000). A large part of the difference in chlorophyll retention between earlier and later-senescing hybrids is expressed in the lower, shaded leaves of the canopy (He et al., 2005; Antonietta et al., 2014), where retention of chlorophyll and photosynthetic capacity might not be utilized fully because of relatively low irradiance. For example, transgenic stay-green lines of tobacco did not show a higher C gain (compared to wild type) because of the respiratory costs associated with delayed senescence of unproductive leaves in shaded parts of the canopy (Boonman et al., 2006).

In Argentina, genetic gains for grain yield between 1965 and 1997 were related to an increase in kernel number, and this was accompanied by increased post-silking accumulation of dry mass and light interception extended up to physiological maturity (Luque et al., 2006). Increased light interception up to physiological maturity suggests that genetic improvement of maize in Argentina also involved a delay of leaf senescence, as reported by Valentinuz and Tollenaar (2004) for North America and Ding et al. (2005) for China. However, recent evidence shows that, in spite of these gains in light interception, grain filling may still be source-limited in modern, high-yielding Argentinean hybrids (Cerrudo et al., 2013), which suggests that it might be advantageous to further delay senescence. Although it seems clear from comparisons of older vs. newer hybrids that genetic improvement over the past decades delayed senescence in maize, growth and yield of contemporary hybrids differing in their senescence behaviour has been studied much less. To test the impact of delayed senescence on grain yield of modern, contemporary hybrids of maize, we compared the dynamics of leaf photosynthetic activity, post-silking growth, and grain yield in four modern hybrids differing in their rates of senescence. Additionally, we estimated the potential photosynthetic contribution of delayed senescence of lower leaves in hybrids with longer persistence of functional leaves.

2. Materials and methods

2.1. Growth conditions

Field experiments were carried out at Estación Experimental Agropecuaria INTA Balcarce, Argentina (37°45' S, 58°18' W)

during the 2004–05 (Exp. I), 2005–06 (Exp. II) and 2006–07 (Exp. III) growing seasons. In Exps. I and II, four maize hybrids (DK682, DK696, AX890 and NK870) were sown in a randomized block design with three replicate plots per hybrid. Previous visual observations showed that canopy senescence occurred earlier in DK682 and DK696 (earlier-senescing) than in AX890 or NK870 (later-senescing), while, at the same time, these four hybrids had similar days to silking and to relative maturity. Therefore, these contemporary, modern hybrids seemed appropriate to compare the impact of delayed senescence on yield and post-silking growth of maize. To estimate the potential photosynthetic gains possibly due to delayed senescence, two hybrids (DK682 and NK870) were chosen for Exp. III because they have similar kernel numbers but contrasting senescence behaviour, based on the results of Exps. I and II. Each plot consisted of four rows, 10 m long and 0.70 m apart. At sowing (October 18, 2004, October 24, 2005 and October 18, 2006), three seeds were placed manually in each hill (0.2 m apart in each row). After emergence, seedlings were thinned to 7 plants m⁻². Plots were fertilized with 30 kg P ha⁻¹ at sowing and 160 kg N ha⁻¹ at the V6 stage (Ritchie and Hanway, 1982) and watered by sprinkler irrigation as needed to prevent water deficits. Atrazine plus acetochlor were applied at pre-emergence to control weeds.

2.2. Leaf area, dry mass and yield determination

Two plants per plot, adjacent in the same row, were harvested at regular intervals for dry mass and leaf area determinations. Plants were separated into stem plus sheaths, leaf laminae, and ears. The green area of each individual leaf blade was measured with a LiCor LI 3100 Area Meter, and all organs were dried at 60°C for at least 4 days until constant weight was achieved.

At physiological maturity, all ears of plants in an area of 4.2 m² in each plot were harvested, threshed and dried at 60°C to constant weight. Yield data were expressed as grain dry weight (g) per m⁻². Mean individual kernel weight (KW) was determined by weighing 1000 kernels per replicate for each hybrid. Kernel number per plant (KNP) was estimated on the basis of KW and grain yield, essentially as in Echarte et al. (2000).

2.3. Ear and leaf N concentration

In Exps. I and II, mature kernels were harvested at physiological maturity, dried, milled to a fine powder and N concentration (% on a dry mass basis) was measured after Kjeldahl digestion. In Exp. III, leaves used for N determination were dried and weighed separately, milled to a fine powder and their N concentration was measured as indicated above.

2.4. Chlorophyll and photosynthesis measurements

To estimate leaf chlorophyll (chl) content, four to five measurements evenly distributed along the length of the leaves from two plants per plot were made with a SPAD 502 Chlorophyll Meter (Minolta, Japan) and averaged. Photosynthesis was estimated from chlorophyll fluorescence measurements (Exps. I, II and III) and through gas exchange analysis with a portable photosynthesis system in Exp. III. A pulse-amplitude modulated FMS2 (Hansatech, UK) chlorophyll fluorometer was used to measure the effective PSII quantum yield of maize leaves in the field, at midday. Care was taken to make measurements on fully illuminated spots of the leaves to estimate photosynthetic capacity at full irradiance. Photosynthetic electron transport rate (ETR) was calculated as in Rosenqvist and van Kooten (2003), except that, to account for the changes in absorbance due to chl loss during senescence, leaf absorbance was estimated from the relationship between absorbance (measured with an integrating sphere; basically as in Long

et al., 1996) and SPAD readings, determined in a parallel set of leaves differing in their senescence stage.

To determine the responses of photosynthesis to irradiance in maize leaves at different stages of senescence, net photosynthesis of DK682 and NK870 was measured in Exp. III at 360 ppm CO₂ and photosynthetically active photon flux density (PPFD) levels ranging from 0 to 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with a CIRAS2 Portable Photosynthesis System (PP Systems, UK). Leaves were enclosed in the leaf assimilation chamber and allowed to acclimate to 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ PPFD provided by a set of LEDs at 25 °C until photosynthesis reached a steady state. Thereafter, irradiance was lowered in steps of 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and photosynthesis was measured at each irradiance level when the steady state was reached (i.e., no change in photosynthesis in two measurements 4–5 min apart). Below 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, measurements were made at 100, 50 and 0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The CO₂ assimilation quantum yield was calculated from the slope of the linear regression between photosynthesis and PPFD between 0 and 400 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. All leaves were light-saturated above 1600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, therefore photosynthesis values at 1600 and 1800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were averaged to calculate light-saturated photosynthesis (A_{sat}).

2.5. Light interception

In Exp. III, light interception profiles were determined (from silking to physiological maturity) at midday on sunny days with an AccuPAR linear (1 m long) light meter (Decagon, WA, USA). Briefly, PPFD was measured 0.5 m above the top of the canopy, and then every 0.25 m starting from the ground. For these measurements the line sensor was placed diagonally covering all the distance between two adjacent rows. To assess possible differences in the profile of light interception between hybrids or due to senescence progression, the profile of light interception across the canopy

was fitted to a linear model including canopy levels up to 90% interception.

2.6. Model-based estimation of photosynthesis

In Exp. III, the expected net photosynthetic rate for the maximum irradiance within the canopy at 0.75, 1.25 and 1.75 m above the ground was estimated using the model described by Dwyer et al. (1995). Percentage transmitted PPFD at different heights within the canopy was fitted to two linear regression models, with a breakpoint at 1 m, except for 81 days after silking (DAS) when a single linear model fitted transmitted PPFD at all canopy levels. Regression equations were used to calculate the percentage of incident PPFD transmitted at 0.75, 1.25 and 1.75 m above the ground at 5, 31, 50 and 81 DAS, and these values were multiplied by 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (which is a reasonable estimate of the maximum incident PPFD at this latitude during the maize grain filling period) to give maximum incident PPFD at different canopy levels.

The following equation was used to calculate net photosynthetic rate at a given irradiance, A_{PPFD} :

$$A_{\text{PPFD}} = \frac{\alpha I}{1 + I[\alpha/(A_{\text{sat}} + D) - (1/2000)]} \quad (1)$$

where α is the quantum yield of net photosynthesis, I = PPFD, A_{sat} is the net photosynthetic rate at light saturation and D is the rate of dark respiration (Dwyer et al., 1995).

The quantum yield of photosynthesis (α), CO₂ exchange at saturating irradiance (i.e., A_{sat}) and dark respiration, i.e., CO₂ exchange calculated by extrapolation to darkness (D), were derived from the photosynthesis-irradiance response curves. There were no significant differences between both hybrids in the α , which remained constant throughout the leaf life cycle at around 0.029. Therefore, this α value was used for the calculation of A_{PPFD} . A_{sat} and D decreased during senescence, however the relationships between

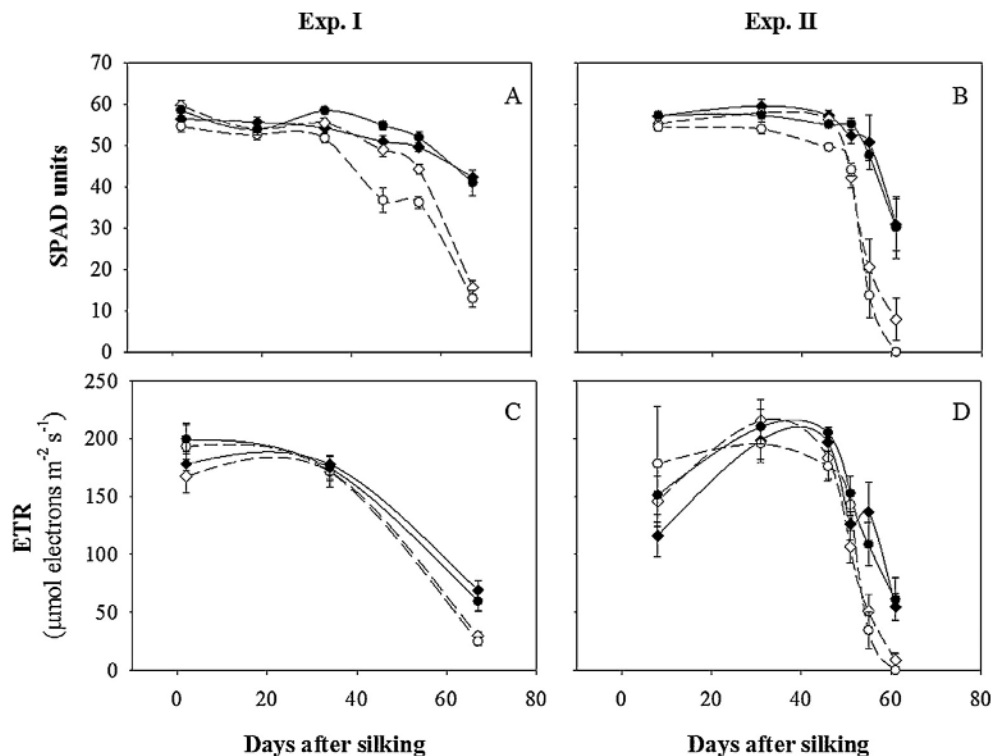


Fig. 1. Changes in the chlorophyll content (SPAD units, panels A and B) and photosynthetic electron transport rate (ETR, panels C and D) during senescence of the ear leaf in Exp. I (A, C) and Exp. II (B, D). Empty symbols and dashed lines denote earlier-senescing hybrids (circles, DK682, diamonds, DK696). Solid lines and full symbols denote later-senescing hybrids (circles, NK870, diamonds, AX890). Vertical bars show the standard error.

A_{sat} or D and SPAD measurements were the same for both hybrids. As inputs in the calculation of A_{PPFD} , average A_{sat} and D values for SPAD readings above or below 35 were used, depending on the senescence stage of the leaf.

The net photosynthetic rate at saturating irradiance for the N% concentration of the leaves (A_{NITROGEN}) was calculated from a linear regression between A_{sat} and leaf N% concentration.

2.7. Statistical analysis

Statistical analysis was carried out using the Statistica 5.1 software (StatSoft, Inc.). Differences were analyzed by analysis of variance (ANOVA) using mixed models and assumptions were checked by Levene's test. For Exps. I and II, DM accumulation, grain yield, KNP, KW and kernel N concentration were analyzed using experiment, hybrids and blocks as fixed factors. For Exp. III, green leaf area per plant, leaf area per canopy level, average SPAD units, leaf N% and ETR were analyzed separately for each sampling date using hybrids and blocks as fixed factors. To assess the significance of linear regressions the F -test was used ($P < 0.05$).

3. Results

The hybrids employed in this study did not differ significantly in the date of silking (i.e., silking occurred at around 90 days after planting), and there were also no differences in their time to physiological maturity (occurring at around 150 days after sowing). Hybrids differed markedly in terms of canopy senescence. In Exps. I and II, most leaves of NK870 and AX890 remained visibly green until grain maturity, while leaves of DK682 and DK696 were completely dry (data not shown).

3.1. Post-silking decline of photosynthetic capacity (Exps. I and II)

In both, Exps. I and II, chl content of the ear leaf started to decrease 30–40 days after silking in DK682 and DK696. In contrast, chl levels decreased much less in NK870 and AX890 (Fig. 1A and B). Chl retention was accompanied by higher rates of photosynthetic electron transport under full irradiance in NK870 and AX890 late in the grain filling period (e.g., 60 days after silking), which was more pronounced in Exp. II (Fig. 1C and D). Thus, NK870 and AX890 showed a delayed loss of photosynthetic capacity (i.e., delayed functional leaf senescence), relative to DK682 and DK696.

3.2. Post-silking dry mass accumulation, grain yield and N concentration in kernels (Exps. I and II)

Delayed leaf senescence in NK870 and AX890 might translate into increased post-silking dry mass accumulation. However, hybrids did not differ in post-silking DM accumulation (Table 1), in spite of the comparatively longer persistence of green, photosynthetically active leaves in NK870 and AX890.

There was no consistent difference in grain yield between earlier and later-senescing hybrids, although grain yield of DK696 was significantly lower than that of the rest of the hybrids (Table 1). Hybrid differences were found for yield components. Kernel number per plant (KNP) was consistently lower in AX890 and DK696. Kernel weight (KW) was higher in AX890 with no significant differences within the rest of the hybrids (Table 1). Hence, for the SG hybrids employed in this work, AX890 and NK870, there was not a consistent trend for yield components associated with the SG trait. In contrast, a consistent trend was found for N concentration in kernels, which was lower in the later-senescing hybrids regardless of a significant experiment \times hybrid interaction (Table 1). In Exp. I, N% in kernels was significantly lower (below 1.19%) in the

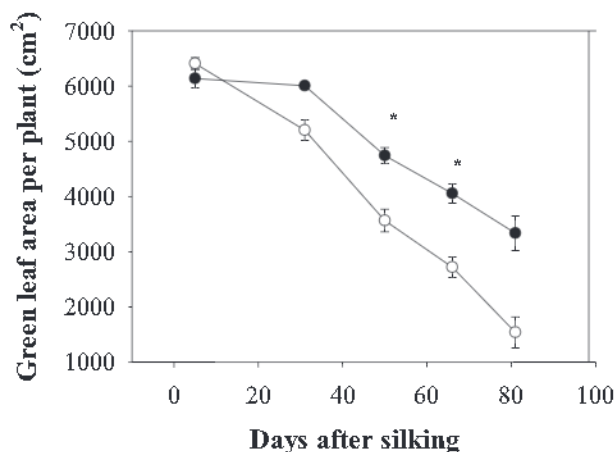


Fig. 2. Green leaf area per plant ($\text{cm}^2 \text{plant}^{-1}$) during the grain filling period. Vertical bars represent the standard error. Asterisks indicate significant differences at $P < 0.05$. Empty symbols denote the earlier-senescing hybrid DK682, while closed symbols denote the later-senescing hybrid NK870.

later-senescing hybrids NK870 and AX890. In Exp. II, hybrid differences remained between the earlier-senescing DK682 and the later-senescing NK870 (Table 1). Interestingly, consistently higher ($P < 0.001$) yields in Exp. II for all hybrids, led to a reduction in N% in kernels in the earlier-senescing hybrids (a dilution in kernel Nc), but to an unchanged (NK870) or even increased (AX890) N% in kernels in the later-senescing hybrids.

3.3. Vertical profiles of leaf senescence (Exp. III)

To understand the impact of the delayed senescence trait at the canopy level, in Exp. III we studied whole canopy senescence in DK682 and NK870, which were chosen as representatives of relatively early and late senescing types, respectively. NK870 and DK682 did not differ in whole plant green leaf area (GLA) at 5 DAS (Fig. 2). The decrease of green leaf area started earlier (5 DAS) in DK682 than in NK870 (31 DAS). Between 31 and 81 DAS, senescence progressed at a higher rate in DK682 ($1.1\% \text{ leaf area day}^{-1}$; calculated as the slope of a linear regression fitted to the data of Fig. 2) than in NK870 ($0.8\% \text{ leaf area day}^{-1}$). Because of the delay in the initiation of senescence, and the lower rate of leaf area loss, NK870 maintained a higher GLA than DK682 at late stages of grain filling. Note that rates of canopy loss in DK682 are similar to those of modern hybrids classified as “stay green” in comparison with older genotypes (Valentinuz and Tollenaar, 2004).

Progression of leaf senescence in different layers of the canopy was characterized by following the changes in green leaf area (GLA) and chl content at 0.25 m intervals above the ground. At 5 DAS, the vertical distribution of GLA was very similar in NK870 and DK682 (Fig. 3A). Thereafter, leaves below 0.75 m started to senesce at a faster rate in DK682 than in NK870, leading to higher GLA at 0.25–0.75 m in NK870 at 31 DAS (Fig. 3C). In the upper part of the canopy, GLA started to decrease after 31 DAS; however, differences between hybrids did not occur before 66–81 DAS (Fig. 3G and I). Bottom-up and top-down senescence resulted in GLA persistence around the ear (1.25–1.50 m above the ground) at 50 and 81 DAS (Fig. 3G and I), particularly in NK870. In summary, NK870 retained a significantly larger GLA during grain filling, but up to 66 DAS this was restricted to the lower part of the canopy (below 0.75 m). After 66 DAS, differences extended to the central leaves of the canopy (1.00–1.25 m) and, since 81 DAS, also to the upper leaves (1.25–1.75 m).

Chlorophyll content of leaves (chl) varied between hybrids, even before GLA differences could be detected (compare Fig. 3A and B).

Table 1

Post-silking dry matter (DM) accumulation, grain yield, kernel number, average kernel weight and kernel N concentration (%) at harvest in the earlier-senescing hybrids DK682 and DK696, and the later-senescing hybrids NK870 and AX890.

Exp	Hybrid	Post-silking DM accumulation (g pl ⁻¹)	Grain yield (g m ⁻²)	Kernel number (m ⁻²)	Kernel weight (mg kernel ⁻¹)	Kernel N concentration (%)
I	DK682	142	1312	4415	297	1.53a
	DK696	150	1067	3702	290	1.37b
	NK870	145	1347	4451	302	1.18c
	AX890	172	1281	3674	348	0.96d
II	DK682	122	1470	4654	316	1.36b
	DK696	111	1322	4668	283	1.26bc
	NK870	149	1510	5086	297	1.14c
	AX890	134	1444	4324	334	1.24bc
	<i>P</i> (Exp)	NS	***	***	NS	NS
	<i>P</i> (Hybrid)	NS	**	**	***	***
	<i>P</i> (E × H)	NS	NS	NS	NS	**

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

At 5 DAS, chl content was slightly higher in NK870 between 0.75 and 1.75 m (Fig. 3B). At 31 DAS, in NK870, all leaves (except for the highest one) contained higher chl contents than DK682. In most canopy layers, and particularly around and below the ear (1.25 m) chl content decreased progressively in DK682 since 5 DAS while in NK870 chl remained almost unchanged up to 81 DAS (Fig. 3J). Therefore, differences between hybrids for chl content at different canopy layers amplified during grain filling. Remarkably, at 50 DAS (and even at 81 DAS), leaves around the ear in NK870 were still non-senescent, showing no change in their chl content.

3.4. Leaf N concentration and photosynthetic electron transport rate of leaves in different positions within the canopy (Exp. III)

To determine if the loss of photosynthetic capacity was delayed at all canopy layers in NK870 relative to DK682, we measured leaf N concentration and photosynthetic electron transport rate (ETR) in basal, mid and upper canopy leaves (Fig. 4). In the earlier-senescing DK682 hybrid, N concentration and ETR in fully illuminated parts of the leaf laminae (i.e., an estimate of photosynthetic capacity) decreased after silking in basal leaves (0.75 m), reaching very low or non-detectable values after 50 DAS (Fig. 4E and F). Compared to the lower layer, at the mid and top of the canopy loss of N was less pronounced, and the decline of ETR started later (31 DAS) in DK682 (Fig. 4A–D). The later-senescing hybrid NK870 maintained higher levels of N at all canopy levels, but particularly at the lower leaves. In NK870, at 0.75 m, N loss was delayed until 66 DAS (Fig. 4E), while for mid (1.25 m) and upper (1.75 m) leaves no apparent N loss was observed up to 81 DAS (Fig. 4C and A). In general, N retention in NK870 paralleled its higher photosynthetic capacity in basal leaves since 31 DAS, while in mid and upper leaves higher photosynthetic capacity was noticed even at 81 DAS (Fig. 4D and B). Thus, photosynthetic capacity persisted longer at all positions within the canopy in NK870, and this was associated with retention of leaf N.

3.5. Light interception (Exp. III)

Delayed leaf senescence might not only increase the photosynthetic capacity of individual leaves, but it might also lead to more efficient light capture by the canopy, particularly at late stages of grain filling. The percentage of light transmitted decreased from the top to the bottom of the canopy (Fig. 5). Progression of senescence between 5 and 50 DAS did not increase the amount of light transmitted to the soil level. Also, in spite of their differences in

green leaf area persistence, there were no differences in transmitted light between hybrids up to 50 DAS (compare data points at 0 cm in Fig. 5A and B). The change in percentage transmitted light throughout canopy layers was also similar at 5, 31 and 50 DAS, and between hybrids (compare slopes in Fig. 5A and B). Differences in light interception could be detected only at 81 DAS, when DK682 and NK870 had lost 76 and 45% of their maximum GLA at silking, respectively (see Fig. 2).

3.6. Changes in quantum yield and in light-saturated CO₂ exchange during leaf senescence (Exp. III)

Clearly, NK870 retained leaf photosynthetic capacity (measured as photosynthetic electron transport rate in fully illuminated spots of leaves) much longer than DK682, but this extended capacity might not be translated into C gain in leaves under limiting irradiance (e.g., at lower canopy levels). Therefore, we studied the CO₂ exchange-PPFD responses of leaves at different stages of senescence to estimate the potential photosynthetic gain from senescence delay in the later-senescing hybrid. The quantum yield of photosynthesis (i.e., the slope of the relationship between CO₂ exchange rate and PPFD below 400 μmol photons m⁻² s⁻¹) was the same regardless of hybrid (0.0293 and 0.0292 for NK870 and DK682, respectively). The quantum yield was not related to the chl content of the leaves, i.e., it did not show significant changes related with senescence (Fig. 6A). In contrast, light-saturated photosynthesis (A_{sat}) was linearly related to leaf N concentration ($r^2 = 0.52$, $P < 0.001$, $n = 20$; Fig. 6B).

3.7. Photosynthesis calculated on the basis of irradiance or leaf N concentration (Exp. III)

Photosynthesis at the average irradiance expected at midday for a sunny day (A_{PPFD}) at 0.75, 1.25 and 1.75 m above the ground, was estimated using the model described by Dwyer et al. (1995) (Eq. (1)). A_{PPFD} increased from the base to the top of the canopy. Since light interception profiles did not vary much between hybrids except at the latest sampling date (Fig. 5), there were no differences in A_{PPFD} between NK870 and DK682 for most of the grain filling period (Table 2). At 50 DAS, A_{PPFD} at 1.75 m was higher in NK870 than in DK682 probably because of maintenance of photosynthetic capacity in the delayed senescence hybrid. Most important, A_{PPFD} of lower leaves retained in NK870 was not very different from DK682, in spite of a large difference in terms of the decline of leaf N, chl and photosynthetic capacity.

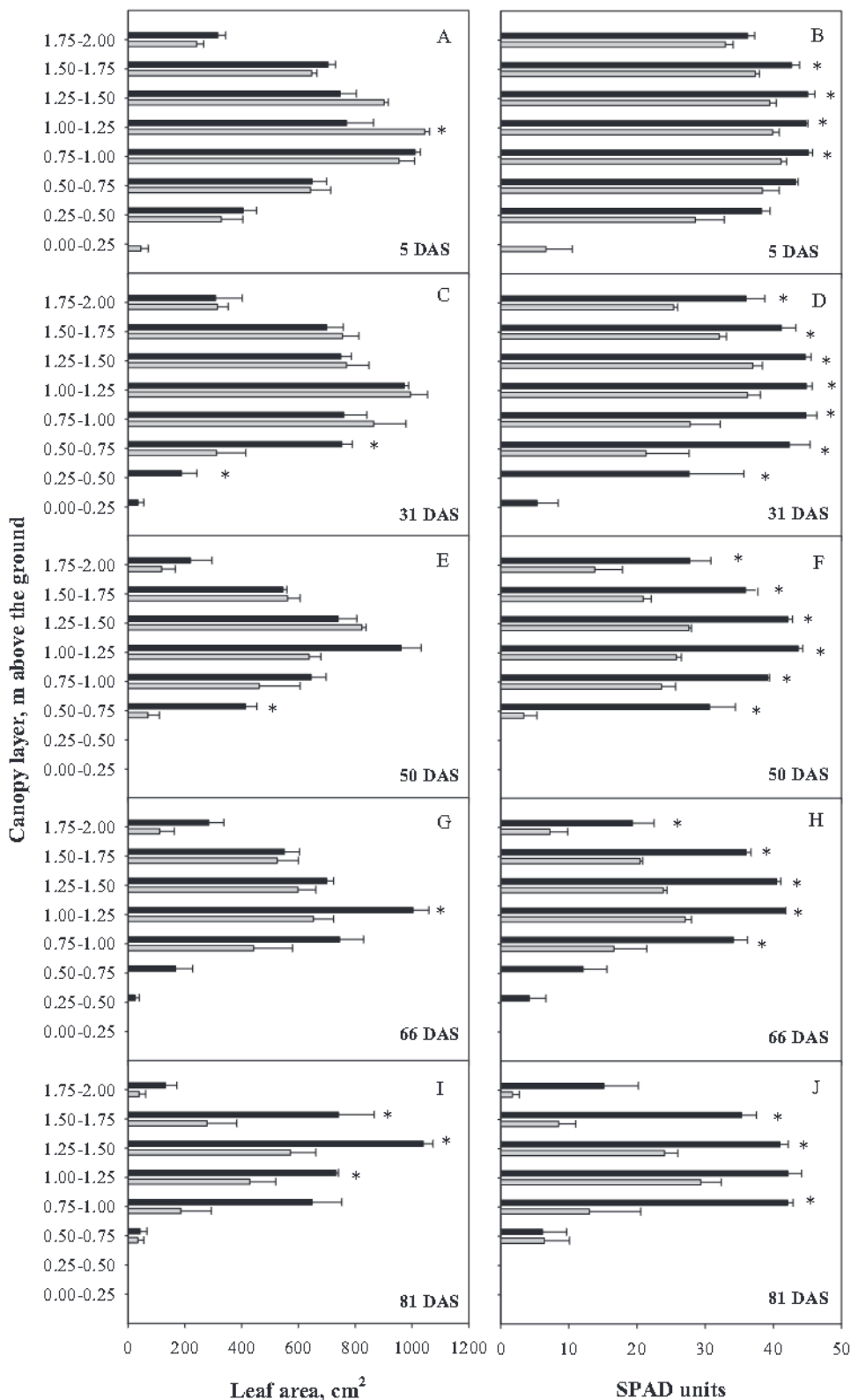


Fig. 3. Vertical distribution of green leaf area (cm², left column), and average chlorophyll content of individual leaves (SPAD units, right column) during the grain filling period in an earlier-senescing (DK682, grey bars) and a later-senescing (NK870, black bars) hybrid of maize in Exp. III. Horizontal bars represent the standard error. Asterisks indicate significant differences between hybrids at $P < 0.05$.

On the basis of data presented above (see Figs. 5 and 6), we analyzed whether the leaves retained at lower canopy levels in NK870 contained higher levels of N than those needed to sustain photosynthesis at those low irradiances. The predicted maximum

leaf photosynthetic activity at saturating irradiance based on leaf N concentration, A_{NITROGEN} , varied in parallel with leaf N concentration. In DK682, A_{NITROGEN} declined faster for the lower leaves (0.75 m) than for the upper leaves (1.75 m), and it remained at

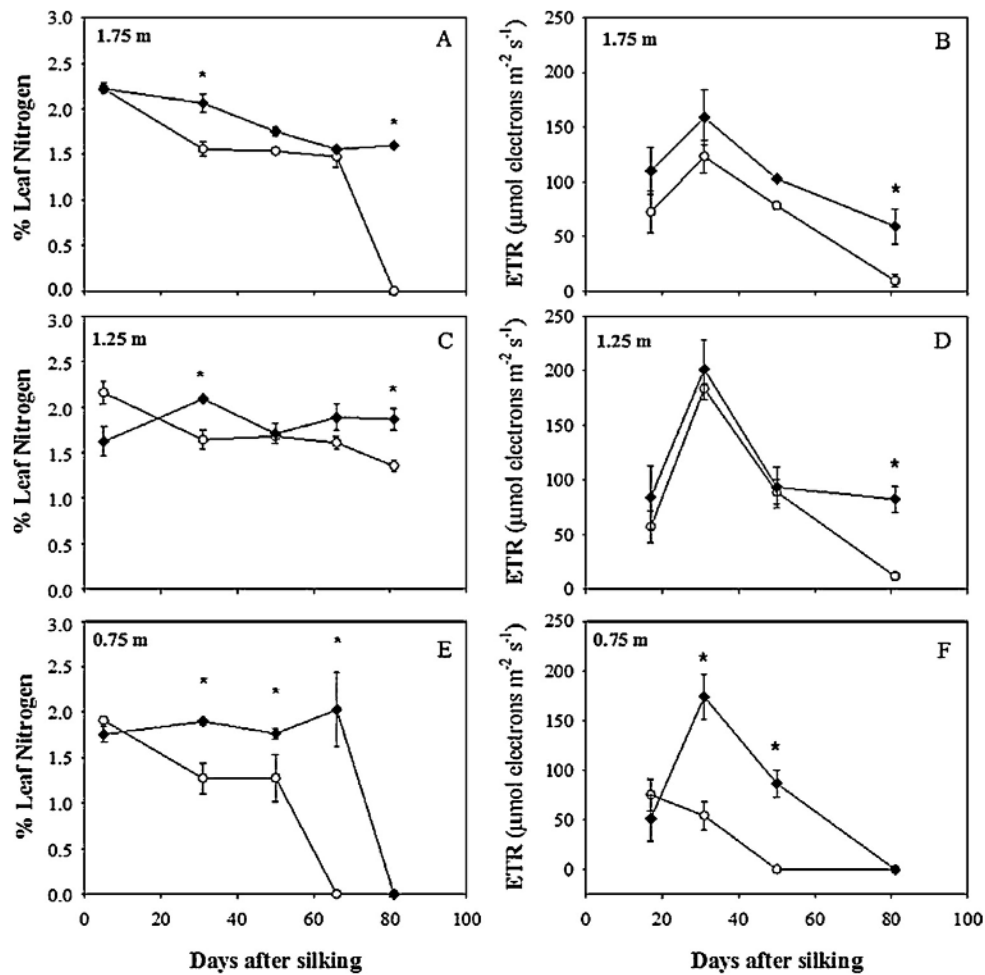


Fig. 4. Changes in leaf N concentration (% leaf Nitrogen, A, C, E) and photosynthetic electron transport rate (ETR, $\mu\text{mole}^{-1} \text{m}^{-2} \text{s}^{-1}$, B, D, F) during the grain filling period in Exp. III. Measurements were made in leaves at different levels above the ground: 1.75 m (A, B), 1.25 m (C, D) and 0.75 m (E, F). Empty symbols denote the earlier-senescing hybrid DK682, and closed symbols denote the later-senescing hybrid NK870. Vertical bars represent the standard error and asterisks indicate significant hybrid differences at $P < 0.05$.

intermediate but relatively constant values for the ear leaf (Table 2). The main difference between DK682 and NK870 was the delayed decline of A_{NITROGEN} in the lower leaves of NK870, which retained higher A_{NITROGEN} even up to 50 DAS. For a leaf at 0.75 m, A_{NITROGEN}

was much higher than A_{PPFD} in NK870 between 30 and 50 DAS, i.e., N retention in the lower leaves led to an excess of N in relation to that needed to sustain photosynthesis at the low irradiances prevailing at the base of the canopy.

Table 2

Photosynthetic activity estimated for the maximum prevailing PPFD at each canopy position (A_{PPFD} , calculated from the model of Dwyer et al., 1995) and photosynthetic activity expected on the basis of leaf N concentration under full irradiance (A_{NITROGEN} , calculated from the fitted equation in Fig. 6B). For all the computations the data utilized were obtained from measurements performed at Exp. III, in leaves at different canopy heights, in different stages during the grain filling period, and for the earlier-senescing DK682 and the later-senescing NK870 hybrids. N.D.: not determined, because very little green leaf area remained in these layers at 81 DAA.

Canopy height	DAS	DK682		NK870	
		A_{PPFD} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	A_{NITROGEN} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	A_{PPFD} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	A_{NITROGEN} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)
0.75 m	5	3.4	22.2	3.03	19.1
	31	2.5	9.3	3.34	22
	50	2.5	0.8	3.39	19.3
	81	N.D.	N.D.	N.D.	N.D.
1.25 m	5	7.9	27.3	10.5	16.4
	31	11.9	16.8	11.2	25.9
	50	5.3	17.5	8	18.2
	81	9.4	11	19.1	21.3
1.75 m	5	17.4	28.5	18.7	28.6
	31	18.6	15.1	18.2	25.3
	50	8.8	14.7	17.5	19.1
	81	N.D.	N.D.	N.D.	N.D.

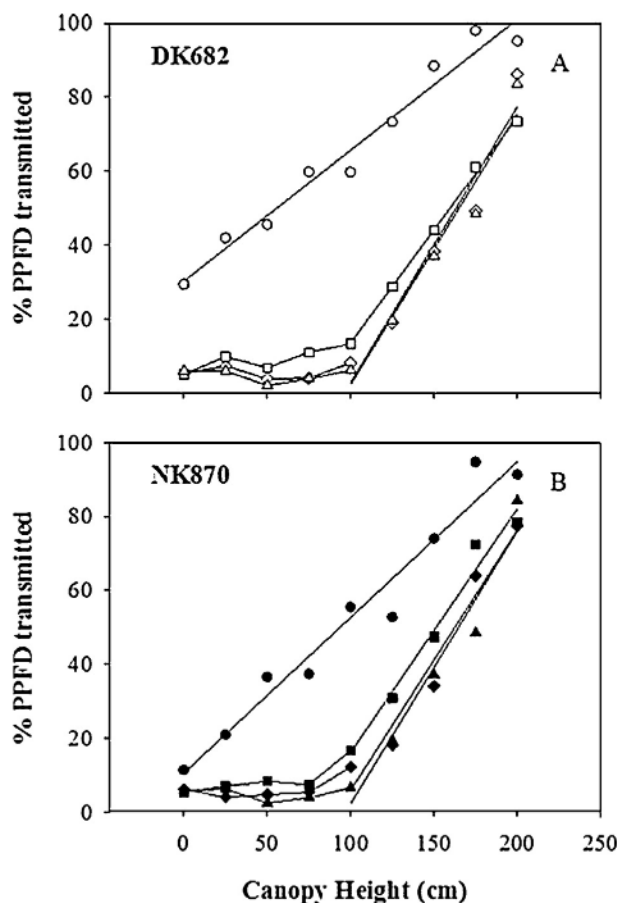


Fig. 5. Percentage of photosynthetically active radiation (PPFD) transmitted at different heights within the canopy. Upper panel, empty symbols: DK682; lower panel, closed symbols: NK870. Measurements were taken at different stages after silking: 5 DAS (diamonds), 31 DAS (squares), 50 DAS (triangles) and 81 DAS (circles). At 5, 31 and 50 DAS, % PPFD transmitted above 1 m was fitted to linear regressions where x represents height. Slopes did not vary markedly between DK682 and NK870 at 5 DAS (74.5 and 70.4, respectively), 31 DAS (61.0 and 65.9), 50 DAS (73.4 and 73.7). At 81 DAS, % PPFD transmitted from 0 to 2 m was fitted to a linear regression, and the slopes were 35.4 for DK682 and 42.0 for NK870.

4. Discussion

4.1. Delay of functional senescence in Argentinean hybrids

There is evidence that genetic gains in yield over the past decades involved the incorporation of delayed senescence, i.e., the ‘stay-green’ trait in North America (Valentinuz and Tollenaar, 2004) and China (Ding et al., 2005). In Argentina in particular, genetic improvement resulted in increased post-silking dry matter accumulation and extended light interception, which suggests a delay of canopy senescence (Luque et al., 2006). There are different types of delayed-senescence genotypes (‘stay-green’) which may differ in the underlying mechanism responsible for senescence delay and in their usefulness for agriculture (Thomas and Howarth, 2000). Some are cosmetic and of no agronomic interest, because they retain chlorophyll and chloroplast membrane proteins in senescing leaves but photosynthetic activity declines more or less in parallel with that of wild type genotypes (e.g., *cytG* of soybeans, Guiamét et al., 1990; *Sid⁸* of *Festuca* sp., Thomas and Howarth, 2000). In contrast, all the delayed senescence maize and sorghum genotypes characterized so far appear to be functional ‘stay-greens’, i.e., the decline of photosynthetic capacity is delayed (e.g., He et al., 2002; Ding et al., 2005). In this study, NK870 and AX890 showed a delay of senescence, expressed not only as chlorophyll retention but also as extended photosynthetic capacity at the leaf level, compared

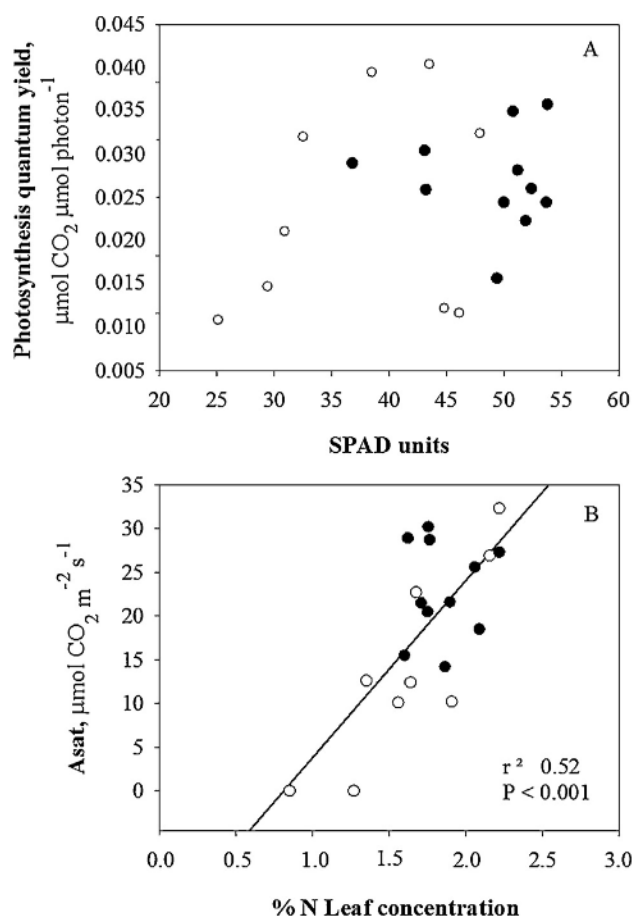


Fig. 6. Photosynthesis quantum yield as a function of chlorophyll content (SPAD units) (A) and light saturated photosynthesis (Asat) as a function of leaf N% concentration (B) measured in Exp. III. Measurements were made in leaves at different canopy positions (0.75, 1.25 and 1.75 m) and at different stages during grain filling (5, 31, 50 and 81 DAS). At 81 DAS, measurements could not be taken in senesced leaves from all canopy layers of DK682 ($n=9$) and from the lower canopy layer of NK870 ($n=11$); as a result, $n=20$ in each regression. Empty symbols denote the earlier-senescing hybrid DK682, and closed symbols denote the later-senescing hybrid NK870.

to DK682 and DK696. Thus, NK870 and AX890 are functional stay green types.

4.2. Delayed senescence did not increase light interception by the canopy up until late stages of grain filling

In addition to extending photosynthetic capacity, delaying the loss of green leaf area might also increase light interception by the canopy. Although the loss of green leaf area started later and proceeded at a slower rate in NK870 than in DK682, integrated canopy light absorption averaged 90–95% in both hybrids between 5 and 50 DAS (when grains had already reached 90% of their final weight, data not shown). The amount of intercepted light (i.e., the reciprocal of transmission) was higher in NK870 only at 81 DAS, close to physiological maturity, and even at this stage canopy interception was 90% in the late senescing NK870, compared to 70% in the earlier-senescing DK682. Much of the green leaf area maintained in NK870 involved the relatively large leaves of the canopy below 1 m, where light interception seems to be saturated at approximately 95% of the incoming PAR (i.e., increases in green leaf area below 1 m have no effect on light interception). Therefore, retention of these leaves did not contribute to increase light interception, whereas retention of upper leaves later during grain filling was also not very important in view of their smaller size. Overall, delayed senescence had

a very small effect on light interception by the crop, and this effect became important only quite late, when grain filling was almost complete.

A substantial part of the delay of leaf senescence in the late-senescing hybrid NK870 involved lower leaves exposed to limiting irradiance. Modelling the predicted photosynthesis of these lower leaves shows that their potential contribution to C gain is quite small (Table 2). However, respiratory costs associated with their maintenance might impose a substantial burden on the C budget of the plant, as shown for a delayed senescence line of tobacco (Boonman et al., 2006). In modern maize hybrids, Antonietta et al. (2014) showed that in a ‘stay-green’ hybrid respiratory rates of lower leaves are maintained unchanged even at late stages of grain filling (500 °C d after silking) while, as a consequence of senescence, respiration drops to zero in an earlier-senescing hybrid. Therefore the net effect on the C balance of retaining lower, shaded leaves might be even counterproductive.

4.3. The delayed-senescence trait may be associated with higher N retention in lower leaves but also with lower N concentration in kernels

In herbaceous plants, the distribution of leaf N along a vertical gradient is partly controlled by the average integrated PPFD incident on each leaf (Drouet and Bonhomme, 1999; Rousseaux et al., 1999), i.e., leaves at the base of the canopy exposed to low PPFD contain lower N concentrations. Lower, shaded leaves start to senesce earlier than other leaves of the canopy (e.g., Sadras et al., 2000), possibly in response to the decline in PPFD and red:far red ratio of incident light (Guamét et al., 1989; Rousseaux et al., 2000). Since ears are the main sink for N after silking, and much of leaf N is redistributed to ears during senescence, delayed senescence and retention of N in leaves might cause a decrease of kernel N concentration if there is not a concomitant increase in N uptake. Previous works showed that the stay-green trait in maize is not necessarily linked with higher post-silking N accumulation (Subedi and Ma, 2005; Antonietta et al., 2014). In those cases, a higher N retention in the lower leaves of delayed-senescence genotypes, such as that found in the present work (Fig. 4) might be partially associated with a lower N concentration in kernels (Table 1).

For leaves at different positions within the canopy and at different stages of grain filling, we calculated maximum photosynthesis at full PPFD for the N concentration of leaves, and photosynthesis at the maximum irradiance that those leaves might experience. Lower canopy leaves of NK870 retained N in excess of that needed to maximize photosynthesis at the low irradiances prevailing at the base of the canopy. Thus, delayed senescence and N retention in lower leaves might represent a penalty for N accumulation in kernels, without positive effects on canopy photosynthesis.

4.4. Photosynthetic performance in stay-greens and its potential impact on grain yield

Maize grain yield may be limited by source activity (Borrás and Otegui, 2001; Borrás et al., 2004), and this limitation may have increased in modern hybrids with a higher number of kernels per plant (e.g., Cerrudo et al., 2013), therefore, delayed-senescence genotypes might out-yield normally senescing types by extending the photosynthetic activity of the canopy. However, in the present work, in spite of the persistence of photosynthetic capacity in NK870 and AX890 compared to the earlier-senescing hybrids DK682 and DK696, there was no consistent advantage of the delayed senescence hybrids in terms of post-silking dry mass accumulation or grain yield. This is in contrast to a number of previous works where the ‘stay-green’ trait is associated with the yield advantage of modern vs. old hybrids (Rajcan and Tollenaar,

1999a; Valentinuz and Tollenaar, 2004; Ding et al., 2005). On the other hand, our results are consistent with those of Antonietta et al. (2014), where the ‘stay-green’ trait did not lead to higher post-silking dry matter accumulation, nor to higher yields within modern Argentinean hybrids differing in senescence behaviour.

In older genotypes, leaf senescence started very early (e.g., in maize hybrids released in the 1950s the photosynthetic capacity of the ear leaf decreased very rapidly starting 2 weeks after silking, e.g., Ding et al., 2005). Also, canopy green leaf area decline was much faster than in modern hybrids (e.g., around 2.85% day⁻¹ for the older, non ‘stay-green’ Pride 5 hybrid released in the 1950s, compared to around 1.40% day⁻¹ for the newer, ‘stay-greens’ Pioneer 3902 and Pioneer 3893 released in the 1980s and 1990s, respectively, Valentinuz and Tollenaar, 2004). Delaying rapid canopy senescence, particularly at the relatively lower planting densities (and therefore greater light penetration within the canopy) typical of previous decades, clearly extended C gain by the crops and, thereby, increased grain yield. In contrast, all the modern hybrids used in the present work, whether relatively earlier- or later-senescing, would be classified as stay green types compared to the behaviour of older hybrids from the 50s or 60s (Valentinuz and Tollenaar, 2004). For example, the rate of green leaf area loss, even in the earlier-senescing hybrid DK682 is still relatively slow (1.1% of plant leaf area day⁻¹) compared to senescence rates reported in previous studies carried out in Canada, both for older and even for modern hybrids (e.g., Valentinuz and Tollenaar, 2004). Thus, in contemporary Argentinean hybrids, differences in post-silking loss of green leaf area between earlier and later-senescing hybrids are smaller than in published comparisons between older vs. newer genotypes (i.e., all our modern genotypes are relatively “stay green”). As a consequence, significant differences in leaf area and light interception occur only towards the end of the grain filling period, when surplus assimilate production might not be converted into a larger grain yield because kernels are probably close to their maximum, final weight.

5. Conclusions

It is clear that over the past decades breeding programmes have delayed maize senescence (e.g., Valentinuz and Tollenaar, 2004; Luque et al., 2006) and this contributed to the increased yield potential of modern hybrids, however, a point may have been reached where further delays of leaf senescence may not necessarily lead to increased grain yield in temperate maize crops under relatively non-stress situations. From our comparison of modern Argentinean hybrids differing in their senescence behaviour it is clear that (1) further delaying senescence may increase light interception very late, when kernels may not utilize this potential surplus of C, (2) most of the delay of senescence involved the lower leaves, where irradiance severely limits photosynthesis and (3) senescence delay (with retention of leaf N) of leaves in basal, poorly illuminated layers of the canopy may represent a penalty on kernel N accumulation. This probably offsets any potential gains due to extended duration of photosynthetic capacity in fully exposed leaves. Overall, this may explain the lack of a consistent increase of grain yield in the later-senescing hybrids used in this study. However, the delayed-senescence trait may still offer a yield advantage under conditions where biotic (e.g., leaf diseases) or abiotic (e.g., water deficit) stresses accelerate senescence and thereby limit photosynthesis and crop growth.

Modern, high-yielding hybrids may be subject to source-limitations even in the absence of abiotic stress (Cerrudo et al., 2013), and increasing C supply in these genotypes might have a positive effect on yield. To meet this need, the delay of senescence should be fine-tuned, both in time and space. It is clear

that maintenance of photosynthetically competent leaves in lower layers of the canopy can be useless (even counterproductive) at the relatively high planting densities normally used nowadays in commercial maize production. Therefore, breeding efforts might be directed towards delaying senescence of leaves around the ear, i.e., genotypes with the top-down bottom-up pattern of senescence (Valentinuz and Tollenaar, 2004). Pommel et al. (2006) and Antonietta et al. (2014) reported on maize hybrids where senescence of lower leaves is not delayed compared to a reference genotype, while senescence of the upper leaves and leaves around the ear is substantially delayed. Thus, it might be possible to breed for hybrids where most of the senescence delay occurs in the more productive mid and upper thirds of the canopy, thereby avoiding N retention in unproductive, shaded leaves. Additionally, later-senescent hybrids with a more erectophile canopy (and, therefore, a lower light extinction coefficient) could increase light penetration to lower leaves and thereby, make persistence of photosynthetic capacity in the lower leaves a productive trait.

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