Maydica

Original paper Open Access

Senescence progression and N yield are related to canopy size at silking in maize hybrids cropped in the Argentinean Pampas

Mariana Antonietta[1]*, Mariano Montenegro[2], Paula Girón[3], Walter Miranda[4], Mirian Barraco[4], Juan J. Guiamet[1]

1 Plant Physiology Institute (INFIVE), CONICET- National University of La Plata, Diag. 113 495, La Plata, Buenos Aires, Argentina

2 Plant Physiology Institute (INFIVE), CONICET- National University of La Plata, Diag. 113 495, La Plata, Buenos Aires, Argentina.
Present address: Crop Protection, FCAyF, UNLP

3 Estación Experimental Agropecuaria General Villegas del INTA. San Martín 26, (6230) General Villegas, Buenos Aires, Argentina.
Present address: MD Corn & Sorghum Placement (CS & Norla), Bayer Crop Science.

4 Estación Experimental Agropecuaria General Villegas del INTA. San Martín 26, (6230) General Villegas, Buenos Aires, Argentina

*Corresponding author: E-mail: antoniettamariana@gmail.com

Keywords: *Maize, senescence, nitrogen, kernels.*

Abstract

Delayed senescence (SG) is considered a desirable trait in commercial maize hybrids. However, the contribution of the SG trait to yield is still controversial and an increasing number of works report penalties over kernel N content. Here, we explore these relationships using a larger number of genotypes than in previous studies and including quantitative appraisals of senescence progression at different canopy layers. Two field experiments were carried out under rainfed conditions at Villegas (12 hybrids) and La Plata (15 hybrids), Buenos Aires Province, Argentina. Delayed senescence was quantified by a novel parameter representing the maintenance of leaf area above critical values (LA_{int}) during the entire grain filling. LA_{int} was mostly dependent on senescence delay at middle and upper canopy layers and it was related to canopy size at silking. The source: sink relationship (estimated as LA_{int}/ kernel number) partially explained the variation in kernel weight but the contribution of delayed senescence to yield was only evident when kernel number was not the main yield determinant. Across experiments, N content in kernels (Ny, kg N ha^{-1}) was related to canopy size at silking (r^2=0.41, n=90, P<0.001). A Principal Component Analysis (PCA) across environments supported a negative relationship between %N in kernels and SG traits, whereas yield and, especially, Ny, were closely related to canopy size at silking. These results provide new insights to understand the impact of the SG trait on yield and N related variables and highlight canopy size at silking as a relevant trait that can impact on senescence progression, yield and N content in kernels.

Abbreviations

LA: Leaf area per plant
LAI: Leaf Area Index
LAint: Integrated leaf area throughout the post-silking period
KN: Kernel number
Ny: N harvested in kernels
PCA: Principal Component Analysis
SG: Stay green
TKW: Thousand Kernel Weight
TT: Thermal time
α: Maximum LA
β1: Rate of LA loss during the first phase of senescence
γ: Onset of the second phase of senescence
β2: Rate of LA loss during the second phase of senescence

Introduction

Maize breeding has delayed canopy senescence, with modern hybrids exhibiting a stay-green trait (SG) compared to previous ones (Duvick 2005). Prolonging the photosynthetic functionality of the canopy may improve post-silking C accumulation while also maintaining photoassimilate supply to roots, which, through extended soil N absorption, may further delay leaf N remobilization and the onset of senescence (Borrell *et*

al. 2001; Li *et al.* 2022). This framework has allowed many seed companies to promote the SG trait as a desirable feature of some commercial maize hybrids. However, the extent to which this assumption is true will depend on environmental limitations (*e.g.*, soil nutrient availability) as well as on the physiological mechanisms leading to the SG phenotype (Antonietta *et al.* 2024). Moreover, several papers show that the SG trait can be expressed regardless of improved post-silking soil N uptake (Antonietta *et al.* 2014, 2016; Liu *et al.* 2021; Shao *et al.* 2021).

Among modern hybrids, delayed senescence does not always bring about a yield improvement. On one hand, this may relate to the fact that under conventional management practices delaying senescence does not contribute substantially to increasing light interception until very late in the crop cycle (Acciaresi *et al.* 2014). On the other hand, many works report lower leaf N remobilization and lower %Nk in SGs compared with earlier-senescing counterparts (Kosgey *et al.* 2013; Acciaresi *et al.* 2014; Antonietta *et al.* 2014, 2016; Swanckaert *et al.* 2017; Zhang *et al.* 2019; Liu *et al.* 2021; Shao *et al.* 2021). Limited N supply for kernels during key stages of grain filling could compromise metabolic pathways, leading to reductions in potential grain weight and yield (Pico *et al.* 2021). Taking together, this raises the question whether breeding efforts towards further delaying canopy senescence should persist in the future.

Ultimately, assessing the impact of canopy senescence on overall crop performance should consider separately the senescence patterns at different canopy layers. Leaf N remobilization occurs earlier in basal leaves (*e.g.*, Chen *et al.* 2016; Antonietta *et al.* 2019) which at the same time, are exposed to very low light intensities and therefore do not contribute substantially to light interception (Acciaresi *et al.* 2014). By contrast, delaying senescence of upper leaves could improve photoassimilate production at late grain filling stages, highlighting the importance of considering differences in senescence related to leaf position.

Most of previous works focused on the impact of the SG trait over yield and %Nk in maize have been based on a reduced number of genotypes (*e.g.*, Kosgey *et al.* 2013; Antonietta *et al.* 2014, Liu *et al.* 2021) and/or do not include a quantitative appraisal of senescence across the canopy (*e.g.*, Fu *et al.* 2020; Hou *et al.* 2021; Chibane *et al.* 2024). To explore whether there is a widespread relationship between the senescence pattern and yield or %Nk among commercial maize hybrids, in the present research we extend these comparisons to a larger number of genotypes (12-15) than in previous works, including a quantitative appraisal of senescence at different canopy layers across two environments. We hypothesized that (i) after silking, maintaining leaf area indexes above critical values will rely mostly on senescence delay at middle and upper canopy layers; (ii) the SG trait will contribute to yield provided leaf area indexes fall below critical values early in the reproductive period; (iii) the SG trait will come at the cost of reduced %Nk and N harvested in grains. Our specific objectives were: (i) to analyse the contribution of different canopy layers to the canopy senescence pattern; (ii) to identify relationships between the senescence pattern and yield; (iii) and between the senescence pattern and %Nk and N harvested in grains.

Materials and Methods

Experimental design

Two field experiments were carried out in 2015-16 (hereafter, Exp. I) and 2018-19 (hereafter, Exp. II). Exp. I was carried out in the Experimental Field of INTA General Villegas (35° 2'00''S; 63°01'00'W), Buenos Aires province, Argentina, located in the Western Pampean Region. The soil is a typical hapludol characterized by a coarse texture with high water permeability and low N retention capacity (Cruzate *et al.* 2023). Twelve commercial maize hybrids recommended for the region (Table 1) were randomly distributed in plots (27.3 m^2), with four rows 0.52 m apart, within three replicated blocks at a final plant density of 8.4 pl m^{-2}. Sowing date was December 3, 2015, following usual practices for the region, where late plantings allow the critical period for yield determination to take place after the driest conditions of January (Otegui *et al.* 2021). Fertilization included 80 kg P ha^{-1} applied as triple superphosphate at sowing and 182 kg N ha^{-1} applied as urea at V6. The crop was rainfed, with conventional weed and pest management. Average silking date for all the genotypes was February 4, 2016.

Exp. II was carried out in the Experimental Field of the Universidad Nacional de La Plata (34°54'24'' S; 57°55'56'' W), Buenos Aires province, Argentina. The soil is a typical argiudol with a fine texture and an underlying layer of expandable clay (Cruzate *et al.* 2023). Fifteen commercial maize hybrids recommended for the region (Table 1) were randomly distributed in plots (19.6 m^2), with four rows 0.7 m apart, within four replicated blocks at a final plant density of 7 pl m^{-2}. However, in some plots there was emergence failure implying that for 6 hybrids there were three replicated plots, and the total number of plots in the experiment was 54. Sowing date was November 22, 2018. Fertilization included 35 kg P ha^{-1} and 31 kg N ha^{-1} applied as diammonium phosphate before sowing and 100 kg N ha^{-1} applied as urea at V7. The crop was rainfed, with

Table 1 - Hybrids included in each experiment, days to silking and relative maturity as reported by the respective seed companies, and senescence descriptors. Integrated leaf area (LA) during the post-silking period (LA_{int}) and parameters obtained from the bilinear regression model applied at the lower, middle, upper and whole canopy level in Exp. I (Villegas) and II (La Plata): α, maximum LA at silking, β1, rate of LA loss during the first phase of senescence, γ, onset of the second phase of senescence, β2, rate of LA loss during the second phase of senescence. Asterisks denote significance: P<0.05 (*), P<0.01 (**), P<0.001 (***) or not significant (NS). Since no hybrid differences were detected for β1 at lower, middle and upper layers, these data are not shown.

	Hybrid	Days to RM	LA int cm^2 °Cd	Canopy				Lower		Middle		Upper	
				α cm^2 pl^{-1}	β1 cm^2 °Cd-1	γ °Cd	β2 cm^2 $°Cd^{-1}$	γ °Cd	β2 cm^2 $°Cd^{-1}$	γ °Cd	β2 cm^2 $°Cd^{-1}$	γ °Cd	β2 cm^2 $°Cd^{-1}$
Exp I	Ax7822	117	301	7789 a	-1.24 ab	400	-21.13	337 abc	-17.96	478	-13.03	610 ab	-22.8
	Cns2093	120	295	7114 abc	-2.76 d	479	-27.92	288 c	-6.96	544	-19.19	566 b	-14.13
	Cns2104	125	283	7095 abc	-2.16 bcd	451	-22.99	327 bc	-10.12	521	-21.06	552 b	-9.74
	Dk190	120	294	7812 a	-2.64 cd	441	-24.54	407 ab	-18.55	520	-15.29	581 ab	-14.53
	DK66	116	312	7885 a	-1.58 abc	445	-20.00	423 a	-14.51	579	-44.93	579 ab	-14.41
	DK70	120	285	7075 abc	-1.71 abcd	417	-22.29	400 ab	-17.58	479	-11.99	566 b	-11.78
	DK72	122	309	7151 abc	-1.58 ab	475	-23.54	389 ab	-9.76	585	-23.97	563 b	-10.92
	DM2738	119	272	6116 c	-1.42 ab	413	-19.26	413 ab	-19.07	493	-12.13	536 bc	-7.23
	Dow505	124	267	6222 bc	-0.81 a	436	-28.85	409 ab	-12.67	499	-22.43	459 c	-9.14
	I797	102	313	7703 a	-1.21 ab	411	-19.98	419 a	-26.86	579	-20.85	664 a	-31.64
	Syn840	122	305	7191 ab	-1.43 ab	438	-20.88	412 ab	-15.87	513	-11.04	580 ab	-11.21
	TJ626	126	310	7950 a	-1.94 bcd	472	-27.06	414 ab	-17.20	598	-25.76	589 ab	-13.68
	P (Hybrid)		NS	*	*	NS	NS	+	NS	NS	NS	*	NS
Exp II	Arg7730	125	490 ab	6869 abc	-1.47 bcd	670	-14.4 a	592 bcde	-4.2 a	706 abc	-8.79 abc	776	-5.54 ab
	Arg7732	125	438 bcdef	6222 bcde	-0.84 ab	577	-15.1 ab	509 f	-4.93 ab	616 c	-10.64 abcdef	764	-9.23 bcde
	Ax7761	117	441 bcdef	5692 de	-0.85 ab	677	-15.9 ab	608 abcd	-6.01 abc	721 abc	-9.25 abcd	803	-7.05 abcd
	Ax7784	117	457 abcde	6742 bc	-1.54 cd	659	-19.6 abc	553 def	-7.79 cd	732 ab	-12.67 cdef	788	-8.94 bcde
	Ax7822	117	481 abc	7083 abc	-0.90 abc	637	-15.6 ab	556 cdef	-7.88 cd	732 abc	-10.65 abcdef	829	-9.34 bcde
	Dk7220	121	409 ef	6383 bcde	-1.96 d	654	-21.7 bc	513 f	-6.6 abc	692 abc	-14.37 f	737	-9.9 cde
	Dk7270	122	465 abcd	7784 a	-1.06 abc	631	-23.5 c	589 bcde	-10.7 e	693 abc	-12.94 def	761	-11.62 e
	Km3916	125	420 cdef	6029 cde	-0.75 a	621	-19.1 abc	562 cdef	-8.35 cde	685 abc	-10.68 abcdef	736	-8.72 abcde
	Kws4500	119	492 ab	7012 abc	-1.01 abc	679	-17.0 ab	651 ab	-10.82 e	784 a	-10.28 abcde	797	-4.77 a
	LT722	122	481 abc	7268 ab	-1.09 abc	687	-24.2 c	615 abcd	-8.09 cd	765 a	-14.17 ef	777	-10.34 de
	Srm566	125	502 a	6666 bcd	-1.32 abc	743	-17.2 ab	675 a	-5.74 abc	774 a	-7.74 ab	803	-6.17 abc
	Srm6600	120	479 abc	6547 bcde	-0.95 abc	698	-21.9 bc	640 abc	-7.57 bcd	723 abc	-11.58 bcdef	794	-10.33 de
	Syn840	123	390 f	5594 e	-0.89 ab	591	-16.3 ab	550 def	-6.6 abc	645 bc	-10.22 abcde	728	-6.76 abcd
	Syn860	122	414 def	6335 bcde	-1.43 abcd	599	-17.8 abc	515 ef	-6.13 abc	628 bc	-9.62 abcd	741	-10.34 de
	Syn875	123	461 abcde	6120 cde	-0.86 ab	652	-14.1 a	634 abc	-9.88 de	720 abc	-6.72 a	807	-4.74 a
	P (Hybrid)		**	**	*	NS	*	***	***	+	*	NS	*

manual and conventional weed and pest management. Average silking date for all genotypes was February 5, 2019.

In both experiments, meteorological conditions were recorded daily by a nearby automated weather station, and thermal time (TT) was calculated using a base temperature of 8°C (Supp. Fig. 1) and expressed as cumulative degree-days during the post-silking period (°Cd).

Senescence progression

At silking, nine (Exp. I) or twenty (Exp. II) plants per genotype from the central rows of each plot were tagged for visual estimations of the senescence pattern. Senescence progression was measured essentially as in Antonietta *et al.* (2014), by non-destructive estimation of individual leaf area based on maximum leaf width and length, and periodical visual records of percentage leaf area senesced (*i.e*, completely yellow and/or visibly dry). Different canopy layers were analyzed separately considering an upper layer (from the topmost up to the second leaf above the ear), a middle layer (from the first leaf above the ear up to the second leaf below the ear) and a lower layer (from the third leaf below the ear up to the bottommost leaf). Senescence progression was adjusted to a bilinear model essentially as in Borrás *et al.* (2003) that identified leaf area (LA) at silking (α), the rate of LA loss during the first phase of senescence (β1), onset of the second (fast) phase of senescence (γ), and the rate of LA loss during the second phase of senescence (β2). Additionally, senescence progression was quantified as the area below the curve formed between LA per plant and cumulative TT, using the trapezoid rule (hereafter, LA_{int}). To avoid misleading values related with supraoptimal canopy size at silking (*i.e*, higher than those needed to saturate light interception), the maximum LA across hybrids was limited to that needed to achieve a leaf area index of 4 (around critical for maize): 4762 cm^2 pl^{-1} at 8.4 pl m^{-2} in Exp. I and 5714 cm^2 pl^{-1} at 7 pl m^{-2} in Exp. II. Thus, genotypic differences in α values were not dragged into LA_{int} calculations

Yield and nitrogen concentration measurements

Yield was obtained by mechanical harvest of the entire plot (Exp. I) or by manual harvest of five plants within the central rows of each plot (Exp. II). Ears were threshed and weighed. A subsample was dried at 60°C to obtain yield at 0% humidity and grains were manually counted to estimate thousand kernel weight (TKW). Kernel number (KN) m^{-2} was obtained based on yield and TKW. The source: sink ratio was estimated as: LA_{int} / KN. For N determinations, kernel samples were homogenised and milled to a powder and then measured following standard protocols using a micro-Kjeldahl automatic analyser (Nade, K9840).

Statistical analysis

Infostat v. 2020 (Di Rienzo *et al.* 2020) was used for statistical analyses and for graphical artwork. Each experiment was analysed separately using mixed models, where hybrids were considered a fixed effect and blocks were considered a random effect. To assess the significance of correlations, the Pearson test was used ($P < 0.05$). Slope differences were tested via dummy variable regression. Principal Component Analysis was done including data from both experiments and considering both experiment and hybrid as classification criteria.

Results

Meteorological conditions varied between experiments. In Exp. I, during de crop cycle (sowing to physiological maturity), mean temperatures were slightly lower (18.6 vs. 20.1 °C in Exp. II) and cumulative rainfall was higher (634 vs. 523 mm in Exp. II) (Supp. Fig. S1). Besides, the vegetative period was shorter in Exp. I (63 days or 846°Cd in Exp. I vs. 75 days or 1026°Cd in Exp. II), which is an expected outcome of the later planting date (Bonelli *et al.* 2016; Otegui *et al.* 2021).

Senescence progression

Senescence progression (*i.e*, loss of green leaf area) is shown in Fig. 1. Overall, onset of fast senescence occurred earlier in Exp. I than in Exp. II (average γ-canopy 440°Cd vs. 650°Cd, respectively). Also, LAI fell below critical values slightly earlier in Exp. I (earlier senescing hybrids showing LAI <4 before 500°Cd) compared to Exp. II. However, when analyzed relative to the duration of the post-silking period in each experiment, LAI remained above critical values for longer in Exp. I than in Exp. II, where LAI started to fall at around 500 °Cd with the senescence process lasting up to 1000 °Cd.

In both experiments, significant hybrid differences were detected for α-canopy and β1-canopy whereas only in Exp. II there were also differences for integrated LA (LA_{int}) and β2-canopy (Table 1). Considering the entire canopy, no-significant differences were detected for the onset of fast senescence (γ-canopy) in none of the experiments. However, when analysing separately the different canopy layers, hybrid effects were detected for γ-lower and γ-upper in Exp. I, and γ-lower, β2-lower, γ-middle, β2-middle and β2-upper in Exp. II (Table 1). By contrast, for each canopy layer, no hybrid differences were detected for β1 (data not shown).

Table 2 - Pearson correlation coefficients (below the diagonal) and P values (above the diagonal) obtained across senescence-related parameters at the whole canopy level or at different canopy layers and dry matter-related and N-related variables in Exp. I (bold letters highlight significant relationships). Integrated leaf area (LA) during the post-silking period (LAint), maximum LA at silking (α), rate of LA loss during the first phase of senescence (β1), onset of the second phase of senescence (γ), rate of LA loss during the second phase of senescence (β2) at the canopy level and at the lower, middle and upper third of the canopy were related to yield, thousand kernel weight (TKW), kernel number (KN m^{-2}) and to N concentration (%Nk) and N yield (kg N ha^{-1}). Relationships with β1 at each layer were not significant and, therefore, were not included in the matrix.

	LA int	αCanopy	β1Canopy	γCanopy	β2Canopy	γLower	β2Lower	γMiddle	β2Middle	γUpper	β2Upper	Yield	TKW	KN	%Nk	N yield
LA int	1	**0.00**	0.75	0.45	0.42	0.71	0.52	**0.01**	0.21	**0.00**	0.05	0.19	0.48	0.20	0.51	0.41
α Canopy	0.83	1	0.34	0.73	0.68	0.94	0.39	0.10	0.27	**0.00**	**0.04**	0.20	0.23	0.15	0.83	0.22
β1 Canopy	-0.10	-0.30	1	0.06	0.32	0.10	0.23	0.60	0.93	0.66	0.62	0.45	0.56	0.80	**0.03**	0.59
γ Canopy	0.24	0.11	-0.56	1	**0.03**	0.39	**0.01**	**0.03**	0.20	0.52	0.21	0.98	0.62	0.70	0.22	0.46
β2 Canopy	0.26	0.13	0.31	-0.62	1	0.34	0.08	0.71	0.87	0.10	0.37	0.80	0.83	0.96	0.14	0.49
γ Lower	0.12	0.02	0.49	-0.27	0.30	1	**0.04**	0.54	0.54	0.96	1.00	**0.01**	0.31	**0.03**	**0.01**	0.43
β2 Lower	-0.21	-0.27	-0.38	0.72	-0.53	-0.60	1	0.92	0.51	0.05	**0.03**	0.11	0.52	0.18	0.27	0.31
γ Middle	0.71	0.50	-0.17	0.62	-0.12	0.20	0.03	1	**0.01**	0.23	0.42	0.09	0.59	0.11	0.85	0.07
β2 Middle	-0.39	-0.35	-0.03	-0.40	0.05	-0.19	-0.21	-0.68	1	0.99	0.90	0.40	0.81	0.56	0.63	0.68
γ Upper	0.79	0.77	-0.14	-0.20	0.50	0.02	-0.57	0.38	0.00	1	**0.00**	0.41	0.59	0.42	0.95	0.38
β2 Upper	-0.57	-0.60	-0.16	0.39	-0.29	0.00	0.63	-0.26	0.04	-0.81	1	0.48	0.59	0.77	0.97	0.45
Yield	0.41	0.40	0.24	0.01	0.08	0.72	-0.49	0.50	-0.27	0.26	-0.23	1	0.08	**0.00**	0.14	**0.01**
TKW	-0.23	-0.37	0.19	-0.16	0.07	-0.32	0.20	-0.17	-0.08	-0.17	-0.17	-0.53	1	**0.00**	0.74	0.08
KN	0.40	0.44	0.08	0.12	-0.02	0.63	-0.41	0.48	-0.19	0.26	-0.10	0.94	-0.78	1	0.30	**0.00**
%Nk	-0.21	-0.07	-0.62	0.38	-0.45	-0.72	0.34	0.06	0.15	-0.02	0.01	-0.45	0.11	-0.32	1	0.42
N yield	0.26	0.38	-0.17	0.24	-0.22	0.25	-0.32	0.54	-0.13	0.28	-0.24	0.74	-0.53	0.78	0.26	1

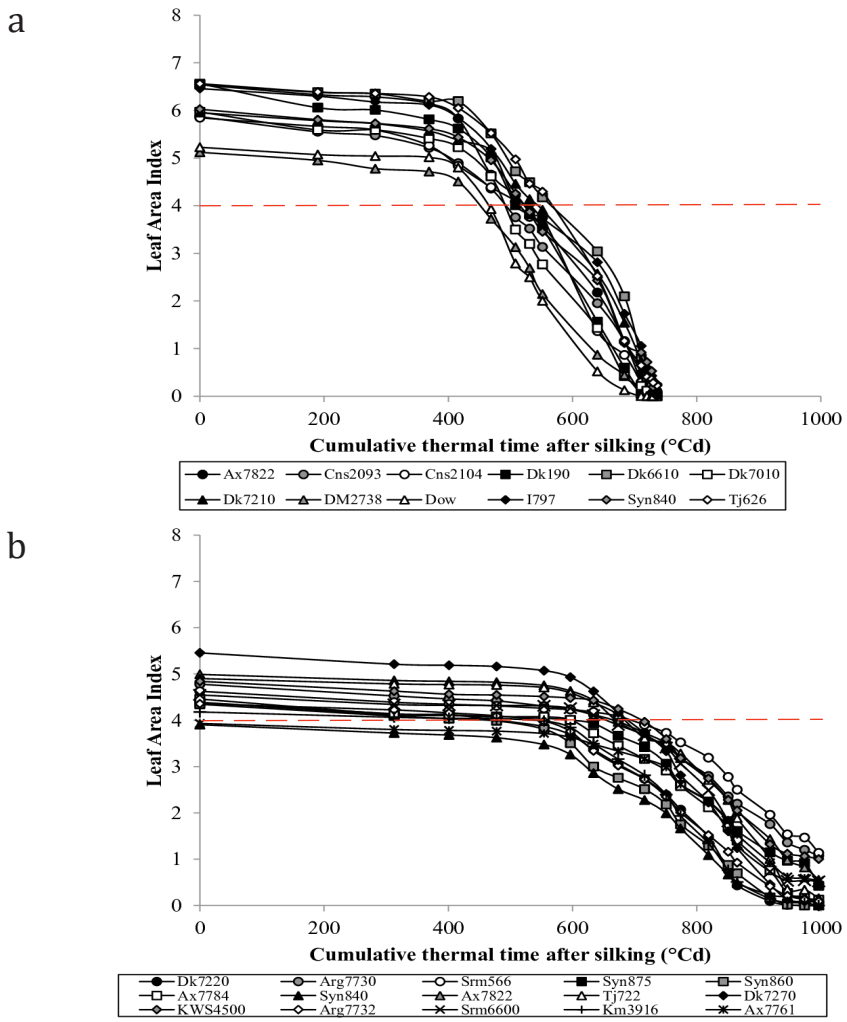


Fig. 1 -Senescence progression from silking up to maturity in Exp. I at Villegas (a, 12 hybrids) and Exp. II at La Plata (b, 15 hybrids). The dashed line represents a standard value of critical leaf area index for maize, above which no further increments in solar radiation interception are expected. This threshold was used for calculating integrated leaf area in the post-silking period.

Among the senescence parameters estimated in this work, LA_{int} is possibly the most comprehensive, as it includes in its value the effects of β1, γ and β2 whereas it considers a maximum α determined by a standard critical LAI of 4 (dashed line in Fig. 1). The relative contribution of the different canopy layers to LA_{int} was explored thorough a correlation matrix (Table 2 for Exp. I and Table 3 for Exp. II). In both experiments, LA_{int} was closely related to γ of the middle (r=0.71-0.77 in Exp. I and II, respectively) and upper layers (r=0.79-0.78 in Exp. I and II, respectively). By contrast, γ of the lower layer only contributed to LA_{int} in Exp. II (r=0.74, Table 3), consistent with the overall lower maximum LAI achieved by the hybrids in that experiment (Fig. 1b). Thus, the relative contribution of the lower layer to the maintenance of a critical LAI depended on the maximum LAI achieved at silking. In addition, LA_{int} was also related to α-canopy (r=0.83 in Exp. I, r=0.66 in Exp. II, Tables 2 and 3, respectively) even though α-canopy values were not introduced in LA_{int} calculations (a single α-canopy required for reaching a critical LAI of 4 was used for all the hybrids).

Across canopy layers, some contrasting patterns were

Table 3 - Pearson correlation coefficients (below the diagonal) and P values (above the diagonal) obtained across senescence-related parameters at the whole canopy level or at different canopy layers and dry matter-related and N-related variables in Exp. II (bold letters highlight significant relationships). Integrated leaf area (LA) during the post-silking period (LAint). maximum LA at silking (α), rate of LA loss during the first phase of senescence (β1), onset of the second phase of senescence (γ), rate of LA loss during the second phase of senescence (β2) at the canopy level and at the lower, middle and upper third of the canopy were related to yield, thousand kernel weight (TKW), kernel number (KN m^{-2}) and to N concentration (%Nk) and N yield (kg N ha^{-1}). Relationships with β1 at each layer were not significant and therefore. were not included in the matrix.

	LA int	αCanopy	β1Canopy	γCanopy	β2Canopy	γLower	β2Lower	γMiddle	β2Middle	γUpper	β2Upper	Yield	TKW	KN	%Nk	N yield
LA int	1	**0.01**	0.84	**0.00**	0.97	**0.00**	0.48	**0.00**	0.55	**0.00**	0.39	**0.00**	0.25	**0.03**	0.73	**0.00**
αCanopy	**0.66**	1	0.46	0.28	0.06	0.39	0.12	0.09	0.13	0.30	0.22	**0.00**	0.14	0.12	0.18	**0.04**
β1Canopy	0.06	-0.21	1	0.44	0.35	0.34	0.27	0.89	0.22	0.39	0.59	0.62	0.43	0.74	0.61	0.85
γCanopy	**0.73**	0.30	-0.21	1	0.46	**0.00**	0.82	**0.00**	0.76	**0.02**	0.32	**0.02**	0.16	0.41	0.46	0.11
β2Canopy	-0.01	-0.49	0.26	-0.21	1	0.95	0.20	0.51	**0.00**	0.31	**0.00**	0.52	0.19	0.48	**0.03**	0.48
γLower	**0.74**	0.24	0.27	**0.82**	-0.02	1	0.21	**0.00**	0.17	**0.02**	0.07	**0.04**	0.59	0.13	0.79	**0.03**
β2Lower	-0.20	-0.42	-0.30	-0.06	0.35	-0.34	1	0.14	0.50	0.51	0.90	0.11	0.29	0.57	0.89	0.17
γMiddle	**0.77**	0.45	-0.04	**0.87**	-0.18	**0.80**	-0.40	1	0.99	**0.00**	0.23	**0.00**	**0.04**	0.34	0.57	**0.01**
β2Middle	0.17	-0.41	0.34	0.09	**0.83**	0.37	0.19	0.00	1	0.22	**0.00**	0.66	0.21	0.38	**0.01**	0.33
γUpper	**0.78**	0.29	0.24	**0.58**	0.28	**0.61**	-0.18	**0.69**	0.34	1	0.23	**0.01**	0.50	0.06	0.87	0.02
β2Upper	0.24	-0.34	0.15	0.27	**0.73**	0.48	0.04	0.33	**0.74**	0.33	1	0.71	0.63	0.32	**0.02**	0.05
Yield	**0.86**	**0.83**	-0.14	**0.60**	-0.18	**0.54**	-0.43	**0.79**	-0.12	**0.67**	0.10	1	0.05	0.05	0.36	**0.00**
TKW	0.32	0.40	-0.22	0.38	-0.36	0.15	-0.29	**0.54**	-0.34	0.19	-0.14	0.51	1	0.07	0.30	0.28
KN	**0.56**	0.42	0.09	0.23	0.20	0.41	-0.16	0.27	0.25	0.50	0.28	0.50	-0.48	1	0.78	0.05
%Nk	-0.10	-0.37	0.14	-0.21	**0.57**	0.07	0.04	-0.16	**0.62**	-0.05	**0.61**	-0.25	-0.29	0.08	1	0.13
N yield	**0.74**	**0.54**	-0.05	0.43	0.20	**0.55**	-0.37	**0.65**	0.27	0.59	0.51	**0.78**	0.30	0.52	0.41	1

Maydica

observed between experiments. In Exp. I, for the whole canopy as well as for each single canopy layer, γ was negatively related to β2, thus delayed onset of senescence was partially compensated for by faster rates of senescence thereafter (Table 2). Besides, onset of fast senescence (γ) was not related between canopy layers implying that hybrids have different strategies, some delaying senescence across the whole canopy (e.g., I797) and others delaying senescence preferentially at the upper layer (e.g., AX7822). By contrast, in Exp. II, there was no significant relationship between γ and β2 within each canopy layer while there was a relationship between γ obtained at different canopy layers (Table 3).

Relationships with yield and yield components

In both experiments, significant hybrid differences were detected for yield and KN m^{-2} whereas only in Exp. II differences in TKW were also detected (Table 4). These variables were included in a correlation matrix to explore their relationship with senescence-related parameters (Table 2 for Exp. I, Table 3 for Exp. II). In Exp. I, yield was mostly explained by KN m^{-2} (r=0.94) implying that it was mainly determined by processes taking place before canopy senescence becomes relevant, and a negative relationship was detected between KN m^{-2} and TKW (r=-0.78) (Table 2). Yield as well as KN m^{-2} were positively related to γ-lower (r=0.72 and r=0.63, respectively) even though this may imply indirect relationships that will be discussed later. In Exp. II, yield was equally determined by KN m^{-2} and TKW resulting in nearly significant relationships with both (r=0.51-0.50, P<0.06, Table 3). Yield was significantly related to LA_{int}, α-canopy, γ-canopy, and γ of the three canopy layers (Table 3). While LA_{int} related to KN m^{-2} (r=0.56), γ-middle was the main parameter contributing to TKW (r=0.54, Table 3).

In both experiments, significant relationships were obtained between the source: sink ratio and TKW (Fig. 2). Consistent with the less evident relationships obtained with senescence parameters in Exp. I (Table 2), TKW was weakly related to the source: sink ratio (r^2= 0.43, P<0.001, Fig. 2a). By contrast, in Exp. II, the lower LAI achieved by silking and the several relationships detected with senescence parameters (Table 3) resulted in a stronger relationship between the source: sink ratio and TKW (r^2= 0.55, P<0.001, Fig. 2b). Besides, the dummy variable analysis detected a significantly higher slope in Exp. II than in Exp. I (264 vs. 154 g of gain in TKW per each unit of increase in the source: sink ratio, respectively, P<0.05).

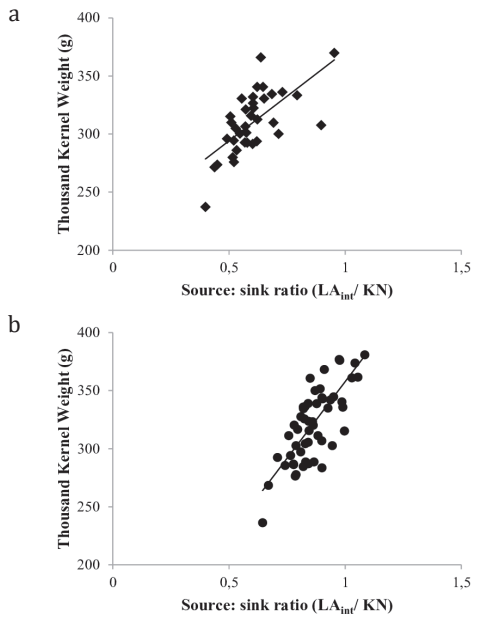


Fig. 2 - Relationships between source: sink ratio (estimated as the quotient between LA_{int} and KN) and kernel weight in Exp. I (a) and Exp. II (b). In each figure, symbols denote replicated blocks and different hybrids. Parameters obtained from the linear regression are: (a) y= 154x + 217, r^2=0.43, P<0.001, n=36; and (b) y= 264x + 93, r^2=0.55, P<0.001, n=54; P<0.001. Significant differences between slopes were detected according to the dummy variable analysis.

Relationships with %Nk and N yield

In both experiments hybrid differences were detected for %Nk and N yield (kg N ha^{-1} in kernels) (Table 4). No significant relationship between %Nk and yield was detected in none of the experiments (Table 2, Table 3), indicating rather mild dilution effects. In Exp. I, %Nk was significantly negatively related to β1-canopy (r=-0.62) and to γ-lower (r=-0.72) (Table 2), implying that faster rates of senescence at early reproductive stages and earlier onset of senescence at the lower canopy layer improved %Nk at harvest. By contrast, N yield was mostly determined by yield (r=0.74), and therefore, also related to KN m^{-2} (r=0.78) (Table 2). In Exp. II, %Nk was positively related to β2-canopy (r=0.57) through β2-middle (r=0.62) and β2-upper (r=0.61) (Table 3), implying that, after the onset of fast senescence, slower rates of senescence related to higher %Nk. As in Exp. I, also in Exp. II N yield was closely related to yield (r=0.78) and, consequently, to yield related variables such as LA_{int} (r=0.74) (Table 3).

However, relationships between senescence parameters and %Nk can be difficult to interpret. In Exp. I, results suggest that delayed senescence penalized %Nk but it could also be the case that SG genotypes had a lower N demand by kernels, allowing senescence to be delayed. On the contrary, in Exp. II, delayed sene-

Table 4 - Yield (kg ha^{-2}) thousand kernel weight (TKW g), kernel number (KN m^{-2}) N concentration in kernels at harvest (%Nk) and N yield (kg N ha^{-1}) in Exp. I (Villegas) and II (La Plata). Asterisks denote significant hybrid differences according to P<0.05 (*). P<0.01 (**). P<0.001 (***) or not significant (NS).

Exp	Hybrid	Yield kg ha^{-1}		TKW g		KN m^{-2}		%Nk%		N Yield kg N ha^{-1}	
Exp I	Ax7822	12417	d	311		3980	c	1.51	cde	189	bcd
	Cns2093	9631	e	329		2940	d	1.84	a	177	cd
	Cns2104	11714	d	312		3797	c	1.78	ab	209	bcd
	Dk190	14435	abc	302		4780	b	1.57	cd	226	ab
	DK66	13311	bcd	317		4195	bc	1.36	e	181	cd
	DK70	12620	d	298		4236	bc	1.39	de	176	cd
	DK72	14811	abc	311		4798	b	1.43	de	211	abc
	DM2738	12491	d	310		4047	c	1.56	cd	195	bcd
	Dow505	12871	cd	327		3931	c	1.5	cde	192	bcd
	I797	14381	abc	324		4452	bc	1.55	cd	223	ab
	Syn840	12109	d	307		3964	c	1.42	de	172	d
	TJ626	15343	a	268		5766	a	1.62	bc	249	a
	P (Hybrid)	***	NS	***	***	**					
Exp II	Arg7730	12443	abcd	293	gh	4244	a	1.53	ab	190	ab
	Arg7732	10718	de	298	fgh	3589	cdef	1.39	bcde	150	cd
	Ax7761	11130	cde	315	def	3533	def	1.39	bcde	156	bcd
	Ax7784	12688	abc	380	a	3336	fg	1.49	abc	188	abc
	Ax7822	13234	a	329	d	4022	abc	1.31	de	174	abcd
	Dk7220	11358	bcde	323	de	3500	def	1.25	e	144	d
	Dk7270	13160	a	325	de	4051	abc	1.31	de	173	abcd
	Km3916	10508	e	358	ab	2934	g	1.43	abcd	152	bcd
	Kws4500	13423	a	355	bc	3777	bcde	1.45	abcd	194	a
	LT722	12600	abc	336	cd	3752	cdef	1.37	bcde	174	abcd
	Srm566	12880	ab	353	bc	3649	cdef	1.37	cde	176	abcd
	Srm6600	11582	abcde	298	fgh	3893	abcd	1.34	cde	155	bcd
	Syn840	9940	e	269	i	3684	cdef	1.51	abc	152	bcd
	Syn860	|10236	e	305	efg	3339	efg	1.51	abc	156	abcd
	Syn875	11787	abcde	279	hi	4227	ab	1.59	a	187	abc
	P (Hybrid)	**	***	***	**	+					

scence was positively related to %Nk which could imply that the SG trait increased soil N uptake, or just that genotypes with a better plant N status were able to delay senescence as well as to achieve higher %Nk at harvest. Thus, N yield can be more informative. Across experiments, the amount of N harvested in grains (N yield) was significantly related to α-canopy (Fig. 3, r^2=0.41, P<0.001) suggesting that in the environments analyzed, N supply for kernels was mostly dependent on the N previously stored in the vegetative canopy.

Data from both experiments were merged into a Principal Component Analysis (PCA) to explore relationships between variables across environments (Fig. 4). To enhance the explanatory power of the model, only LA_{int} and canopy senescence parameters were included alongside yield, KN m^{-2}, %Nk, and N yield; TKW was excluded due to its negligible contribution to the overall variation. The two-dimensional biplot illustrates the projection of the dataset onto the first two principal components, which accounted for 71.3% of the total variance. Yield was highly correlated with KN m^{-2} but showed an orthogonal (uncorrelated) relationship with senescence parameters. By contrast, canopy senescence parameters were inversely related to %Nk, implying either a trade-off between the SG trait and %Nk or a causal relationship where constitutively lower %Nk allows the SG trait to be expressed. Consistent with the relationship obtained in Fig. 3, in the PCA, N yield was closely related to α-canopy.

Discussion

Senescence progression

Onset of fast senescence (γ) started earlier in Exp. I compared to Exp. II (mean γ-canopy 440°Cd vs. 652°Cd, respectively, Table 1). This might be an outcome of the later sowing date in Exp. I which tends to contract both the vegetative period and the effective grain filling period (Bonelli *et al.* 2016). Moreover, the larger KN m^{-2} achieved in Exp. I also reduced the source: sink ratio (ranging between 0.39-0.95 in Exp. I and between 0.64-1.08 in Exp. II, Fig. 2), which may, as well, have promoted an earlier canopy senescence (Borrás *et al.* 2003; Abeledo *et al.*, 2020).

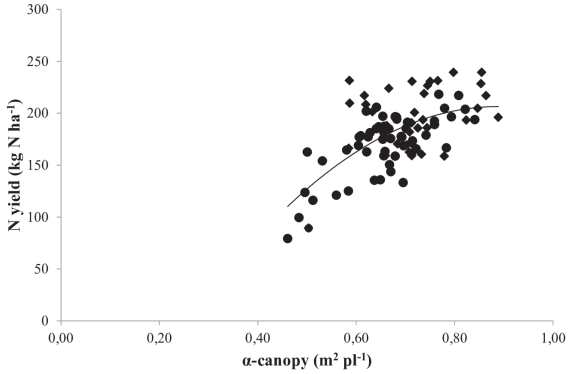


Fig. 3 - Polynomial regression between α-canopy (representing canopy size at silking) and the total amount of N harvested in grains at physiological maturity (N yield) across experiments. Each symbol represents a plot in Exp. I (full diamonds) or Exp. II (full circles). Parameters obtained from the equation are: y=(-525) x^2 + 933x -208; r^2=0.41.

In any case, in both experiments senescence advanced progressively across the canopy, with average γ values increasing from 386, 532 and 570°C in Exp. I, and 584, 708 and 772 °Cd in Exp. II, for the lower, middle and upper layers, respectively (Table 1). This acropetal pattern of senescence progression is usually reported in field-grown maize crops and has implications regarding the relative involvement of different canopy layers on specific developmental processes. Under low N supply, earlier senescence taking place at the lower canopy layer improves %Nk at early stages reducing grain abortion rates (Liu *et al.* 2021) and increasing endosperm cell number and potential kernel weight (Pico *et al.* 2021). By contrast, onset of senescence at the middle or upper canopy layers occurs later and will relate mostly (if it does at all) to TKW. Thus, as senescence proceeds across canopy layers, different physiological processes will be taking place, highlighting the need to consider the impact of senescence in each layer separately.

Regarding genotypic differences, in Exp. I, similar onset of fast senescence at the canopy level involved differences at specific canopy layers (Table 2). Some hybrids delayed senescence consistently across the canopy while others expressed a SG phenotype only at the upper layer which has been related to higher yields at high planting density (Antonietta *et al.* 2014; Xu *et al.* 2024) and N deficiency (Antonietta *et al.* 2016; Liu *et al.* 2021). By contrast, in Exp. II, onset of fast senescence took place later and γ values were related among layers, suggesting comparatively milder environmental pressure and stronger ontogenetic control over the senescence process.

To our knowledge, this is the first research aiming to describe senescence progression throughout the reproductive cycle as well as across the canopy in a relatively large number of genotypes. Other works assessing the contribution of the SG trait to yield have either been based on comparisons of two genotypes (e.g., Zhang *et al.* 2019; Liu *et al.* 2021), or when using a larger number of genotypes, their approach to senescence progression is limited. Among these, some have used qualitative rankings (e.g., Kosgey *et al.*, 2013), measurements over a single leaf (Fu *et al.* 2020; Hou *et al.* 2021; Chibane *et al.* 2021, 2024), and/or at specific stages of the crop cycle (e.g., at harvest, Shao *et*

Maydica

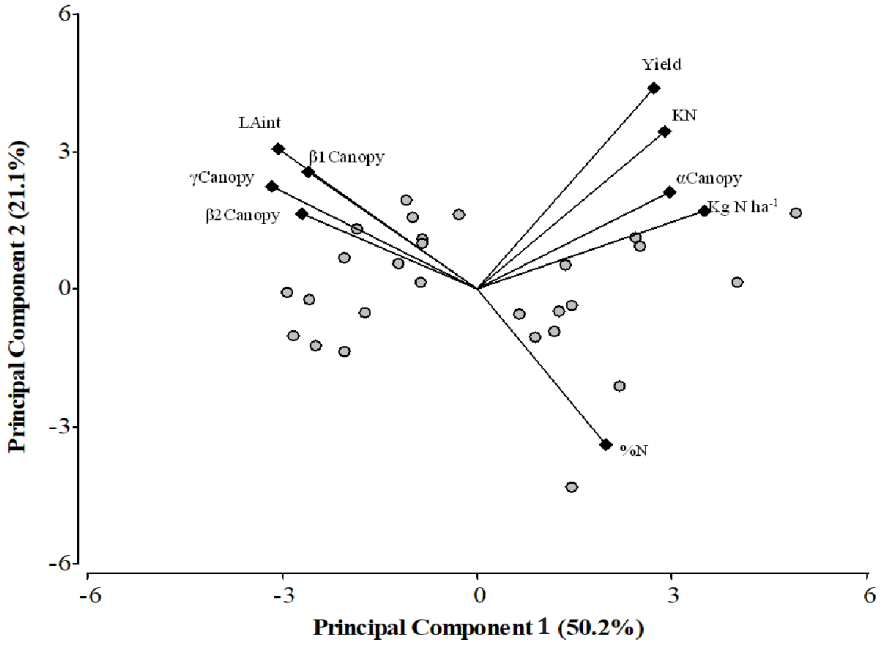


Fig. 4 - Principal component analysis including data from both experiments for canopy senescence parameters and yield and N variables (black diamonds); grey circles represent different hybrids. The following variables were included: integrated leaf area in the post-silking period (LA_{int}), canopy size at silking (α-canopy), rate of senescence during the first, slow, stage of senescence (β1-canopy), onset of the second, rapid, stage of senescence (γ-canopy), rate of senescence during the second stage of senescence (β2-canopy), yield (kg ha^{-1}), kernel number (KN m^{-2}), N concentration in kernels at harvest (%N) and N yield (kg N ha^{-1}).

al. 2021; at 45 days after silking, Chibane *et al.* 2024), just to mention few. In our work, the LA_{int} parameter allowed us to capture differences in LAI late in the cycle while avoiding overestimations related to supraoptimal LAI at silking.

Overall, our results support the idea that a large canopy size achieved at silking (represented by α-canopy) can delay senescence progression thereafter (LA_{int}, Table 2, Table 3). In sorghum, a larger canopy size has also been associated with the SG trait under limited N supply (Hou *et al.* 2021), whereas under post-silking drought the SG trait seems to be the outcome of a smaller canopy reducing water consumption in the vegetative period (George-Jaeggli *et al.* 2017). In our work, a larger canopy may have implied a larger source of remobilizable N for growing kernels, alleviating the pressure on leaves that senesce later on, which is also supported by the relationship between α-canopy and N harvested in grains (Fig. 4). In contrast, reducing canopy size by defoliation treatments accelerates senescence, which could partially be the outcome of decreasing the source of remobilizable N at the canopy level (Abeledo *et al.*, 2020). In any case, the role of a large canopy size contributing to delay senescence will depend on the environment, with possibly larger advantages under limited soil N supply.

Yield and yield components

Our results reinforce the idea that when KN is the main yield determinant (as occurred in Exp. I), the impact of the SG trait may be negligible, whereas environments or management conditions that impose a source limitation in the reproductive period could allow the advantages of the SG trait to be expressed (as in Exp. II). In addition, the relationships obtained between the source: sink ratio and TKW showed a significantly lower slope in Exp. I (Fig. 2), that is consistent with the decreased photothermal quotient experienced by crops in late sowings (Bonelli *et al.* 2016; Otegui *et al.*, 2021). Thus, even under source limitations, some environmental conditions may determine a rather null contribution of the SG trait to yield.

The fact that different plant densities were used between experiments (8.4 pl m^{-2} vs. 7 pl m^{-2} in Exp. I and II, respectively) also deserves some discussion. Plant densities used in maize crops at moderate and high yielding

environments often lead to supraoptimal LAI at silking (as occurred in Exp. I, Fig. 1a). This implies that even if genotypes show differences in senescence progression (Exp. I, Table 1) these will not be translated into advantages in terms of critical LAI (no differences in LA_{int} in Exp. I, Table 1) or light interception until late in the crop cycle (Acciaresi *et al.* 2014). By contrast, if lower plant densities are used (as in Exp. II), differences in senescence progression will promptly determine differences in LA_{int}, improving the source: sink ratio, TKW (Fig. 2b) and yield (Table 3). Thus, the advantages of the SG trait could be larger under low planting densities or in cold environments that limit achievable LAI. For example, in the Southern Pampas, with average planting densities around 6.5 pl m^{-2} and PAR interception below 95% at silking, increasing N input beyond farmer's levels delayed senescence and improved TKW and yield (Moises *et al.* 2024). Similarly, yield advantages of SG varieties have been reported at 6 pl m^{-2} (Chibane *et al.* 2021).

N concentration in kernels and N yield

Many previous works comparing maize hybrids with contrasting senescence behaviour show lower %Nk of SGs compared with earlier-senescing counterparts which usually also implies lower amounts of N harvested in grains (Kosgey *et al.* 2013; Antonietta *et al.* 2014; Shao *et al.* 2021). Whether this reflects a lower N demand by kernels (allowing senescence to be delayed) or a low N supply from leaves is not clear. In Exp. I, senescence parameters representing early reproductive stages (β1-canopy, γ-lower), were negatively related to %Nk but not to Ny, which was mostly determined by yield. Since Exp. I was carried out under a high N input (180 kg N ha^{-1}) and %Nk achieved at harvest was also high (1.54% on average), these results may reflect a trend towards lower maximum %Nk in SGs reinforcing findings from other works (Antonietta *et al.* 2016; Zhang *et al.* 2019; Shao *et al.* 2021).

By contrast, in Exp. II, %Nk as well as Ny were positively related to senescence delay (Table 3) but onset of fast senescence occurred much later, with a mean γ-lower of 584°Cd (Table 1) which is around 46 days after silking. Kernel N accumulation follows a sigmoidal pattern, stagnating after 30- 45 days after silking (e.g., Ning *et al.* 2017; Yin *et al.* 2025). Thus, when senescence takes place after this period, no significant contribution of leaf N remobilization to kernel N may be expected. Instead, the relationship found may be evidence of the influence of plant N status (indirectly, %Nk) on senescence rate and therefore, photoassimilate supply (e.g., Moises *et al.* 2024). Since post-silking N accumulation was not measured in our work, we cannot discard the possibility that senescence delay contributed to post-silking N uptake and N in kernels, as reported when senescence delay was achieved through benzyladenine sprayings to lower leaves (Liu *et al.* 2022).

Across experiments, a consistent relationship was detected between Ny (N harvested in grains) and α-canopy (Fig. 3), highlighting the importance that maximum canopy size can have as a N source for growing kernels. In line with this, even after a high initial fertilizer input of 240 kg N ha^{-1}, available soil N (as NO_3—N) at silking can be as low as 2 mg kg^{-1} particularly in coarse textured soils (Feng *et al.* 2016). These conditions may also arise in soils with a long agricultural history because of nutrient mining and soil organic matter depletion (Sainz Rozas *et al.* 2019). In these environments, the ability for an early N capture in leaves during the vegetative period may determine N supply for kernels in the reproductive period as well as the final N harvested in grains.

Conclusion

In this work we provide evidence that maintaining LAI above critical levels is mostly dependent on senescence delay at middle and upper canopy layers. Our results also show that even though the SG trait can improve the source/sink ratio and TKW, its overall contribution to yield depends on the relative importance that KN m^{-2} has on yield determination. Consistently across experiments, canopy size at silking was closely related to senescence progression and to N content in kernels at harvest. Further work is needed to unravel causal relationships regarding N supply and demand in SG genotypes

Statements and declarations

The authors have no competing interests to declare that are relevant to the content of this article

References

Acciaresi HA, Tambussi EA, Antonietta M, Zuluaga MS, Andrade FH, Guiamét JJ (2014) Carbon assimilation, leaf area dynamics, and grain yield in contemporary earlier-and later-senescing maize hybrids. Eur J Agr 9: 29-38. *https://doi.org/10.1016/j.eja.2014.05.007*

Antonietta M, Acciaresi HA, Guiamet JJ (2016) Responses to N deficiency in stay green and non-stay green Argentinean hybrids of maize. J Agr Crop Sci 202: 231-242. *https://doi.org/10.1111/jac.12136*

Antonietta M, Fanello DD, Acciaresi HA, Guiamet JJ (2014) Senescence and yield responses to plant density in stay green and earlier-senescing maize hybrids from Argentina. Field Crops Res 155: 111-119. *https://doi.org/10.1016/j.fcr.2013.09.016*

Antonietta M, Girón P, Costa ML, Guiamét JJ (2019) Leaf protein allocation across the canopy and during senescence in earlier and later senescing maize hybrids, and implications for the use of chlorophyll as a proxy of leaf N. Acta Phys Plant 41: 1-10. https://doi.org/10.1007/s11738-019-2943-5

Antonietta M., Martinez D, Guiamet, JJ (2024) Delayed senescence and crop performance under stress: always a functional couple? J Exp Bot 75: 4244-4257.

Abeledo LG, Savin R, Slafer GA (2020) Maize senescence under contrasting source-sink ratios during the grain filling period. Env Exp Bot 180: 104263.

Bonelli LE, Monzon JP, Cerrudo A, Rizzalli RH, Andrade FH. (2016) Maize grain yield components and source-sink relationship as affected by the delay in sowing date. Field Crops Res 198: 215-225.

Borrás L, Maddonni GA, Otegui ME (2003) Leaf senescence in maize hybrids: plant population, row spacing and kernel set effects. Field Crops Res 821: 13-26. https://doi.org/10.1016/S0378-4290(03)00002-9

Borrell A, Hammer G, Van Oosterom E (2001) Stay-green: A consequence of the balance between supply and demand for nitrogen during grain filling? Ann App Biol 138: 91-95. https://doi.org/10.1111/j.1744-7348.2001.tb00088.x

Chen Y, Wu D, Mu X, Xiao C, Chen F, Yuan L, Mi G (2016) Vertical distribution of photosynthetic nitrogen use efficiency and its response to nitrogen in field-grown maize. Crop Sci 56: 397-407.

Chibane N, Caicedo M, Martinez S, Marcet P, Revilla P, Ordás B (2021) Relationship between delayed leaf senescence (stay-green) and agronomic and physiological characters in maize (*Zea mays* L.). Agronomy 11: 276.

Chibane N, Revilla P, Yannam VRR, Marcet P, Covelo EF, Ordás B. (2024) Impact of irrigation, nitrogen fertilization, and plant density on stay-green and its effects on agronomic traits in maize. Front Plant Sci 15: 1399072.

Cruzate GA, Morras HJM, Pizarro MJ, Gómez LA (2023) Argentina físico-natural: Suelos. ANIDA. Atlas Nacional Interactivo de Argentina. Instituto Geográfico Nacional. [01/05/2024]. https://static.ign.gob.ar/anida/fasciculos/fasc_suelos.pdf

Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat versión (2020) Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

Duvick DN (2005) The contribution of breeding to yield advances in maize (*Zea mays* L.). Adv Agr 86: 83-145. https://doi.org/10.1016/S0065-2113(05)86002-X

Feng G, Zhang Y, Chen Y, Li Q, Chen F, Gao Q, Mi G (2016) Effects of nitrogen application on root length and grain yield of rain-fed maize under different soil types. Agron J 108: 1656-1665.

Fu W, Wang Y, Ye Y, Zhen S, Zhou B, Wang Y.,.Huang Y (2020) Grain yields and nitrogen use efficiencies in different types of stay-green maize in response to nitrogen fertilizer. Plants 9: 474.

George-Jaeggli B, Mortlock MY, Borrell AK (2017) Bigger is not always better: reducing leaf area helps stay-green sorghum use soil water more slowly. Env Exp Bot 138: 119-129. https://doi.org/10.1016/j.envexpbot.2017.03.002

Hou X, Xue Q, Jessup KE, Zhang Y, Blaser B, Stewart BA, Baltensperger DD (2021) Effect of nitrogen supply on stay-green sorghum in differing post-flowering water regimes. Planta 254: 63.

Kosgey JR, Moot DJ, Fletcher AL, McKenzie BA (2013) Dry matter accumulation and post-silking N economy of 'stay-green'maize (*Zea mays* L.) hybrids. Eur J Agr 51: 43-52. https://doi.org/10.1016/j.eja.2013.07.001

Li R, Hu D, Ren H *et al.* (2022) How delaying post-silking senescence in lower leaves of maize plants increases carbon and nitrogen accumulation and grain yield. The Crop J 10: 853-863.

Liu Z, Hu C, Wang Y, Sha Y, Hao Z, Chen F, Yuan L, Mi G (2021) Nitrogen allocation and remobilization contributing to low-nitrogen tolerance in stay-green maize. Field Crops Res 263: 108078. https://doi.org/10.1016/j.fcr.2021.108078

Moisés Dominguez C, Andrade FH, Monzon JP, Reussi Calvo NI, Cerrudo AA (2024) Nitrogen deficiency in maize fields of the Southern Pampas does not affect kernel number but reduces weight per kernel. Field Crops Res 312: 109394.

Ning P, Fritschi FB, Li C (2017) Temporal dynamics of post-silking nitrogen fluxes and their effects on grain yield in maize under low to high nitrogen inputs. Field Crops Res 204: 249-259.

Otegui ME, Riglos M, Mercau JL (2021) Genetically modified maize hybrids and delayed sowing reduced drought effects across a rainfall gradient in temperate Argentina. J Exp Bot 72: 5180-5188. https://doi.org/10.1093/jxb/erab139

Pico LBO, Zhang C, Vyn TJ (2021) The central role of ear nitrogen uptake in maize endosperm cell and kernel weight determination during the lag period. Field Crops Res 273: 108285. https://doi.org/10.1016/j.fcr.2021.108285

Rozas HS, Eyherabide M, Larrea G, Cuesta NM, Angelini H, Calvo NR, Wyngaard N (2019) Relevamiento y determinación de propiedades químicas en suelos de aptitud agrícola de la región pampeana. In Actas

Simposio Fertilidad. FERTILIZAR Asociación Civil, Rosario (pp. 141-158).

Shao H, Shi D *et al.* (2021) Nutrient accumulation and remobilization in relation to yield formation at high planting density in maize hybrids with different senescent characters. Arch Agr Soil Sci 67: 487-503. *https://doi.org/10.1080/03650340.2020.1737678*

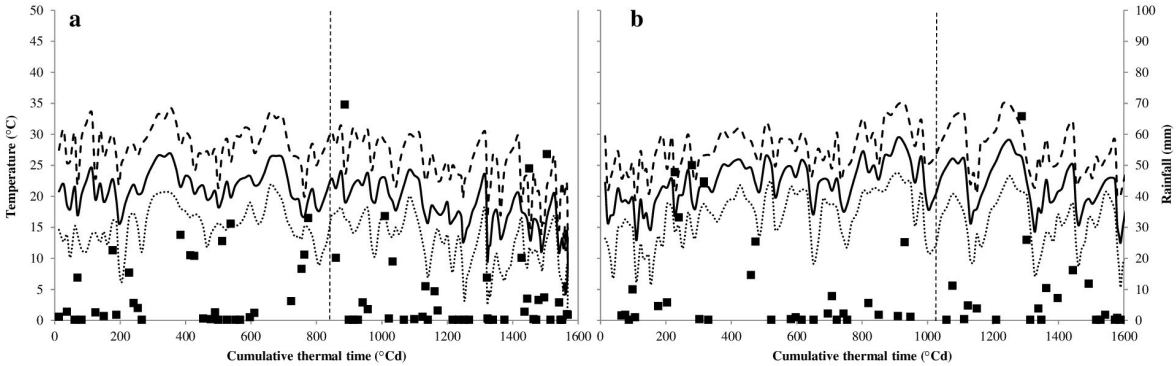
Swanckaert J, Pannecoucque J, Van Waes J, Steppe K, Van Labeke MC, Reheul D (2017) Stay-green characterization in Belgian forage maize. J Agr Sci 155: 766-776. *https://doi.org/10.1017/S002185961600085X*

Xu T, Wang D *et al.* (2024) Plant Growth Regulators Enhance Maize (*Zea mays* L.) Yield under High Density by Optimizing Canopy Structure and Delaying Leaf Senescence. Agronomy 14: 1262.

Yin PJ, Wang XL, Wu YW, Liu F, Tao Y, Liu QL, ... Yuan JC (2025) Effects of nitrogen fertilizer on protein accumulation in basal-middle and apical kernels of different low nitrogen tolerant maize hybrids. Front Plant Sci 16: 1526026.

Zhang LL, Zhou XL *et al.* (2019) Post-silking nitrogen accumulation and remobilization are associated with green leaf persistence and plant density in maize. J Integr Agric 18: 1882-1892.

Supplementary Material



Supplemental Figure S1 - Meteorological conditions during the experiments at Villegas (a) and La Plata (b). Maximum temperature (dashed lines), mean temperature (solid lines) and minimum temperature (dotted lines) and daily rainfall records (full squares) throughout the crop cycle. The vertical dashed line indicates silking date for all the hybrids studied in each experiment (February 4, 2016 in Exp. I. February 5, 2019, in Exp. II).



Maydica's Editor
Dr.ssa Carlotta Balconi
CREA - Research Centre for Cereal and Industrial Crops
Via Stezzano, 24 24126 Bergamo - ITALY
carlotta.balconi@crea.gov.it



Maydica's Technical Editor
Jacopo Barone
j.barone@masaf.gov.it

crea
Consiglio per la ricerca in agricoltura
e l'analisi dell'economia agraria