LEAF SENESCENCE AND THE PROFILE OF EXPANDED LEAF AREA IN MAIZE (*Zea mays* L.)

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ABSTRACT

LEAF SENESCENCE AND THE PROFILE OF EXPANDED LEAF AREA IN MAIZE (*Zea mays* L.)

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University of Guelph, 2002

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Leaf senescence is a major factor affecting light interception and dry matter accumulation during the grain-filling period. Reduced leaf senescence has been associated with maize yield genetic improvement. The objectives of this study were i) to quantify maize senescence in terms of visual and functional symptoms in older and newer hybrids, and ii) to examine the area-per-leaf profile in maize plants grown under a range of environments, agronomic practices and hybrids. Greater green leaf area throughout the grain-filling period in newer compared to older hybrids was the result of a greater leaf area index at silking and a delayed onset of visual leaf senescence. In addition, functional symptoms of leaf senescence (i.e., a decline in carbon exchange rate, stomatal conductance and chlorophyll fluorescence) were apparent in leaves that stayed green during later phases of the grain filling period. A bell-shape function proved to be a robust mean of quantifying the vertical leaf area distribution in maize. Variations due to year, nitrogen, plant density, hybrid, and row spacing on total leaf area could be quantified by measuring their effects on the area and position of the largest leaf. Overall, this study suggests that further advances in maize yield improvement will likely occur by combining 'stay green' with the maintenance of high photosynthesis through the season rather than extending green leaf area duration *per se*. Furthermore, the study

demonstrates that the profile of area per leaf profile of maize is conservative trait that could be exploited to make more rapid and precise estimations of both leaf area and senescence.

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CHAPTER I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Meeting the global requirements for food and feed grains for the next decades represents a huge challenge that will require a coordinated effort of breeders, physiologists and agronomists. Nevertheless, they have faced similar challenging periods through the last century with successful results. For instance, whereas world population increased 50% between the late 1960s and the early 1990s (Evans, 1993), average yield of each of three staple cereal crops, rice, wheat and maize, increased by even more than that (Miflin, 2000). The tremendous increase in crop yield during the last century was achieved through advances in agronomy and plant breeding (Miflin, 2000). Fertilizer use and effective crop protection have marked the advances in agronomy, and cultivars and hybrids with increased grain yield have marked the advances in plant breeding. However, although agronomic factors could contribute to future crop yield increases, the greater increase in crop yield will come mainly from genetic improvement (Stuber et al., 1999; Miflin, 2000). Thus, the study of the physiological basis of yield improvement during the past could identify avenues of past yield improvement and also indicate avenues for future yield improvement (Tollenaar and Wu, 1999).

Physiological basis of yield improvement

The physiological bases of grain yield improvement have been studied in most grain crops including wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), soybean (*Glycine max* L.), and rice (*Oryza sativa* L.) (Slafer et al., 1994; Tollenaar, 1989a; Ferreres et al., 1986; Morrison et al., 1999; Peng et al., 2000). The results of these studies divide most crops into two groups. The first group includes crops such as barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.), wheat and sunflower. Higher yields in this group have mainly been associated with increased harvest index, without any increase in total seasonal dry matter accumulation (Cattivelli et al., 1994; Peltonen-Sainio, 1994; Slafer et al., 1994; Ferreres et al., 1986). In the second group, which includes temperate maize and rice, high yield has been generally associated with increased seasonal dry matter accumulation (Tollenaar et al., 1994; Ashraf et al., 1994).

In temperate maize, the contribution of genetic improvement to the overall yield improvement has been estimated to be between 40 and 100 % (Derieux et al., 1987; Tollenaar, 1989a; Duvick, 1992). Between 1958 and 1988 grain yield of maize grown in Ontario increased 1.7% per year and about 85% of that improvement resulted from increases in total season above-ground dry matter accumulation (Tollenaar, 1989a). Most of the observed differences in dry matter accumulation between older and newer hybrids occurred during the grain-filling period (Tollenaar, 1991; Tollenaar and Aguilera, 1992).

Greater dry matter accumulation in more recent hybrids could be the result of an improved ability to capture and use resources (Tollenaar and Wu, 1999). Improved resource capture represents an increased crop efficiency to access resources provided by the environment, such as solar radiation, water and nutrients, whereas improved resource use reflects an enhanced crop efficiency to convert the resources captured to grain yield. Since both capture and conversion efficiency of radiation are the ultimate determinants of post-silking performance of maize crop in temperate regions, it would appear that leaf senescence represents a serious loss of potential grain yield. Loss of chlorophyll and decline of photosynthesis are two ordered events occurring during leaf senescence and represent the most documented symptoms of leaf senescence (Nooden, 1988). Thus, from its consequences on both capture and use of light during the last part of the growing season, senescence represents a natural and essential target in any attempt to increase maize yields in temperate regions.

Leaf senescence and light capture

It has been know for a long time that major differences in crop yield can be due to differences in the duration of photosynthetic activity (Watson, 1952). During senescence, leaves show a decline in a wide range of physiological functions (Thomas and Stoddart, 1980) that, once integrated throughout the day and the growing season, would affect the crop photosynthetic performance.

A green and closed canopy during the major part of the growing season is essential to maximize light capture. Characteristics like fast leaf expansion and low leaf angle at early growth stages and delayed leaf senescence (i.e., *stay-green*) at late crop life cycle are factors most commonly associated with improved light capture (Thomas and Howarth, 2000). Fast leaf-area expansion accelerates light absorption and, consequently, the rate of dry matter accumulation per unit land will be increased during the early part of the season. Similarly, horizontally oriented leaves result in improved light capture with lower leaf area index during early stages of development (Duncan, 1971). However, grain yield improvement in maize is more closely associated with the ability to maintain greater absorptance (i.e., delayed leaf senescence) and, consequently, accumulate more dry matter in late stages of development. Tollenaar and Aguilera (1992) reported that greater grain yield in a more recent hybrid was associated more with dry matter accumulated during post-silking stages than pre-silking stages and greater grain yield in more recent hybrids has been also associated with longer *stay-green* (Duvick, 1992).

Nevertheless, in spite of its contribution to improved light capture, *stay -green* does not always seem to be related to increases in dry matter and grain yield. Indeed Thomas (1987) found a decline in photosynthesis in fescue (*Festuca pratensis*) mutants whose leaves remained green until maturity. The poor relationship between leaf chlorophyll and photosynthesis, and probably dry matter accumulation, indicates that not only light capture but also light use must be considered when yield improvement is being analyzed. Based on the distinct relationship between chlorophyll and photosynthesis, Thomas and Smart (1993) described four different types of *stay-green*. One of them, defined as "cosmetic" *stay-green*, is characterized by retained pigmentation with a decline in photosynthesis. Although modern hybrids of maize have not been categorized by type of *stay green*, research supports the contention that some degree of "cosmetic" *stay-green* might be found in modern maize hybrids (Smart et al., 1995). For example, a comparison between two *stay green* hybrids showed differences in net photosynthesis during the last two to three weeks of the grain-filling period (Connell et al., 1987; Crafts-Brandner et al., 1984a). Tollenaar and Bruulsema (1988) reported differences in dry matter accumulated after silking in two *stay-green* hybrids with high and stable light capture. This was consistent with a report by Tollenaar and Aguilera (1992) that showed differences in dry matter accumulation even when the proportion of light capture by the canopy did not differ between hybrids. Since most modern hybrids of maize already possess *stay green* and, consequently a maximized light capture from silking to maturity, studies examining light use (i.e., photosynthetic performance) and senescence could help to clarify the physiological basis of yield improvement and be important as a guide to future gains.

Leaf senescence and light use

In order to study the relationship between leaf senescence and light use, an evaluation of photosynthetic performance is required. The estimation and measurement of photosynthetic performance has been a permanent target in crop physiology studies and a general understanding has been reached from growth analysis, an approach that primarily focuses on rate of change in crop dry weight and leaf area. Thus, by using crop growth rate as an estimate of net photosynthesis, strong associations with grain yield have been observed (Hawkins and Cooper, 1981; Tollenaar et al., 1992). Great advances in measuring leaf and canopy photosynthesis have been possible due to the availability of instruments quantifying carbon exchange rate. Measurements of both canopy and leaf photosynthesis have been frequently compared. For example, determinations of canopy photosynthesis during the season have shown high correlation with grain yield (Puckridge, 1971; Wells et al., 1982; Ashley and Boerma, 1989), but disadvantages such as facilities to enclose the crop canopy and time spent in taking measurements limit the number of genotypes that can be compared. On the other hand, although the measurement of leaf photosynthesis is relatively simple, variations due to leaf age, leaf position, leaf angle and intercepted radiation require extensive sampling before an integrated value of crop photosynthesis can be obtained (Earl, 1998). If high rates of leaf photosynthesis could be sustained throughout all levels of crop organization (i.e., canopy, day and growing season) and if these are paralleled by high rates of dry matter accumulation, grain yield could be improved by increasing leaf photosynthesis. More recently, chlorophyll fluorescence has been shown to be an easy, rapid, non-destructive field technique to evaluate the light reactions in photosynthesis (Bolhar-Noremkampf et al., 1989). Basically, the chlorophyll fluorescence technique relies on the fact that light energy absorbed by chlorophyll has three competitive destinations (photochemistry, heat dissipation and fluorescence emission) and changes in photochemistry and heat dissipation are reflected in changes of chlorophyll fluorescence. The use of chlorophyll fluorescence to estimate the photosynthetic performance of crops grown in the field was suggested by Bilger et al. (1995) and successful results were reported by Earl (1998). Unfortunately, the series of variables derived from parameters measured by chlorophyll fluorometers are frequently hard to interpret. However, a better understanding of the parameters involved in photosynthesis can be achieved by combining chlorophyll fluorescence with gas exchange measurements (Maxwell and Johnson, 2000).

Photosynthetic performance of maize through the season has been examined in various experiments. Peters et al. (1974) used canopy photosynthesis to compare hybrids representing five decades, and found that old low yielding hybrids showed a faster rate of photosynthesis decline between anthesis and physiological maturity. More recently, a delay in the decline of leaf photosynthesis during the grain-filling period has been reported in newer maize hybrids compared to older hybrids (Ying et al., 2000). Differences in photosynthetic performance between newer and older hybrids have been shown to be greater when maize is grown under stress conditions such as high plant density (Tollenaar, 1992), drought (Nissanka et al., 1997), low soil N (Rajcan and Tollenaar, 1999) or low temperature (Ying et al., 2000).

Leaf senescence may also be associated with a decline in leaf photosynthesis during periods of high incident radiation during cloudless days. Diurnal depressions of photosynthesis have been reported in rice (Ishihara and Saito, 1987), soybean (Huck et al., 1983) and sunflower (Quick et al., 1992) during periods of maximum light in cloudless days.

Depressions in photosynthesis rate at the afternoon of cloudless days have been also found in maize. Bunce (1990) reported a lower photosynthesis in the afternoon than the morning for the same level of radiation despite more favorable temperature for photosynthesis of maize in the afternoon. A similar finding was reported more recently by Hirasawa and Hsiao (1999). Diurnal depression in photosynthesis could be greater as leaf senescence progresses as a result of changes occurred at chloroplast level (Woolhouse, 1984). As the growing season progresses, changes in number, structure, and function of chloroplasts result in a reduced ability to cope with high levels of light and, consequently, photosynthesis may be inhibited by an excess of light as leaf senescence progresses. Such an inhibition, termed photoinhibition, has been reported in a wide number of species (Ögren, 1994; Long et al., 1994; Ögren, 1988; Hayden et al., 1986), and the magnitude of photoinhibition depends on genotype (Murchie et.al., 1999; He et al., 1996), stress level (Ögren, 1988; Ludow and Powles, 1988) and canopy characteristics (Murchie et al., 1999). Even though the agronomic significance of photoinhibition is hard to quantify, between 10 and 40% of carbon gain could be lost due to photoinhibition on cloudless days (Ögren and Sjostrom, 1990; Long et al., 1994) and could be associated with stress in upper leaves of maize plants grown at very low density (Tollenaar, 1992). Because of the likelihood that photoinhibition increases in parallel with senescence (Kar et al., 1993), potential daily losses in photosynthesis from this process would increase as growing season advances.

Rationale and Objectives

Physiological traits resulting in increased capture and a more efficient use of light during the grain-filling period have been responsible for enhanced maize yield in temperate regions. Premature loss of chlorophyll, the most common visual symptoms of leaf senescence, has been related to yield differences between older and newer maize hybrids, but little is known about functional symptoms of leaf senescence. In this study, visual and functional symptoms, and the vertical profile of leaf senescence between older and more recent maize hybrids were investigated to examine the nature of more efficient capture and use of light.

We hypothesize that the general reduction in crop growth rate in the maize plant during the grain-filling period is associated with leaf senescence which may result in a reduced ability of maize plants to sustain high levels of photosynthesis, even under favorable seasonal and daily conditions, which would result in lower than expected rates of dry matter accumulation during the grain-filling period.

Specifically, this study involved two sets of experiments whose respective objectives were i) to quantify maize senescence in terms of visual and ii) functional symptoms of leaf senescence in older and newer hybrids. A third set of experiments was carried out to examine the area-per-leaf profile in maize hybrids grown under a range of environments and agronomic practices.

CHAPTER II

CHANGES IN VISUAL SYMPTOMS OF LEAF SENESCENCE ASSOCIATED WITH MAIZE GRAIN YIELD IMPROVEMENT

ABSTRACT

Grain yield improvement of maize (*Zea mays* L.) hybrids is associated with delayed leaf senescence. A study was conducted to compare the temporal and spatial progress of visual symptoms of leaf senescence in an old (Pride 5) and two more recent maize hybrids (Pioneer 3902 and Pioneer 3893). The visual symptom of leaf senescence (VS), defined as the loss of greenness, was rated on each individual leaf of plants growing in the field at 1, 3.5, and 12 plants m^{-2} near Elora, Ontario, during three growing seasons. The greater green leaf area during the grain-filling period in newer vs. older hybrids was the result of greater maximum leaf area index, delayed onset of VS, and low rates of visual leaf senescence. Plant density affected differentially the progress of VS of hybrids during the second half of the grain-filling period. The rate of VS during the second half of the grain-filling period was correlated more with changes in stover weight between silking and maturity than with grain yield. A profile of leaf senescence where leaves positioned at the top and bottom third of the plant showed faster progress of senescence than leaves placed in the central third, was observed in two higher yielding growing seasons and was more marked in the newer hybrids. This study confirms that the better productivity of more recent hybrids during the grain-filling period results from a delay in VS and suggests that the top-bottom profile of leaf senescence represents an ideal pattern of leaf senescence for high yield.

INTRODUCTION

Senescence represents an endogenously controlled degenerative process that leads to death (Leopold, 1975). During senescence leaves lose their greenness as a result of a decline in chlorophyll content, providing a clear visual symptom of leaf senescence. Delayed appearance of visual symptoms of leaf senescence or *stay green* has been associated with the improved performance of more recent maize hybrids in North America (Crosbie, 1982; Tollenaar, 1991; Duvick, 1997). For instance, between 1958 and 1988 grain yield of maize hybrids grown in Ontario has increased as 1.7% per year and about 85% of that improvement was associated with increased total above-ground dry matter accumulation (Tollenaar, 1989a). The greater dry matter accumulation of newer vs. old hybrids has been the result of increased dry matter accumulation during the grain-filling period (Tollenaar and Aguilera, 1992), which has been associated with delayed appearance of visual symptoms of leaf senescence (Rajcan and Tollenaar, 1999).

The progress of visual symptoms of leaf senescence during the grain filling-period may vary as a result of water and nitrogen stress and /or source:sink ratio, i.e., the ratio between assimilate supply and the potential of the grain to accommodate assimilates (Tollenaar, 1977). The early appearance of visual symptoms of leaf senescence due to water and nitrogen stress has been reported by Wolfe et al. (1988) and Uhart and Andrade (1995). The source:sink ratio during the grain-filling period is an important factor in the regulation of leaf senescence at the whole-plant level (Tollenaar and Daynard, 1982). Maize hybrids with an improved source capacity may stay green until late in the season (Rajcan and Tollenaar, 1999) which constitutes an advantage with respect to those hybrids that show early symptoms of leaf senescence (Hageman and Lambert, 1988). Sadras et al. (2000) found that accelerated leaf senescence was associated with assimilate accumulation as a consequence of reduced grain set. Tollenaar and Daynard (1982) and Thomas and Smart (1993) indicated that leaf senescence may be accelerated as a consequence of both starvation or excess accumulation of assimilates. Thomas (1992) defined a window between an upper and lower threshold of accumulated assimilates and suggested that as long as a leaf is within this window, senescence is not accelerated. Thomas's concept could be useful in the analysis of differences in rate of senescence in maize when reproductive sinks are totally eliminated by ear removal or when grain set is reduced. Extreme variation in the source sink-ratio has resulted in large contrasts between genotypes. Crafts-Brandner et al. (1984b) found that one of the three hybrids they studied did not show accelerated leaf senescence even though assimilate accumulation was evident as a consequence of ear removal. A less drastic way to alter source:sink ratio can be performed by changing plant density. Andrade et al. (1999) found that sink size was more affected than source when plant density was increased. Thus, the relationship between leaf senescence and alterations in the source:sink ratio could be studied by varying plant density.

A profile of maize leaf senescence progressing from the bottom leaves up, as well as from the top leaves down, resulting in leaves centered around the ear remaining green longest has been observed under both controlled environment and field conditions (Tollenaar and Daynard, 1978; Wolfe et al., 1988) and is exacerbated under water and nitrogen stress, or ear removal (Wolfe et al., 1988). In temperate maize, where yield is generally limited by assimilate supply, the presence of the top-bottom leaf senescence profile might be thought of as a significant limitation to increases in crop productivity.

Indeed, although the top-bottom leaf senescence profile has been mainly identified from visual observation, studies where photosynthesis rates at different leaf positions were measured also suggest the existence of a functional top-bottom leaf senescence profile. Thiagarajah et al. (1981) reported that center leaves maintained a high photosynthesis rate for a longer period than leaves positioned above and below the center leaves. A similar result was reported in a study that included six maize hybrids (Dwyer et al., 1989).

To the best of our knowledge, the top-bottom leaf senescence profile has not been examined in terms of genetic improvement (i.e., older vs. more recent maize hybrids. The objective of this study was to examine the temporal and spatial progress of visual symptoms of leaf senescence in an older (Pride 5) and two more recent maize hybrids (Pioneer 3902 and Pioneer 3893) grown across a wide range of plant densities.

MATERIALS AND METHODS

Cultural practices and experimental design

The study was conducted during the 1999, 2000 and 2001 growing seasons at the Elora Research Station, Ontario (43° 38'N, 80° 25'W, 380 m above sea level). The soil type is a London loam soil (Aquic Hapludalf, USDA taxonomy) with tile drainage and an organic matter content of 3.8-4.0%. Between 1 May and 10 October, the average precipitation in the region is approximately 400 mm and average seasonal heat unit accumulation is around 2650 CHU (Crop Heat Units; Brown and Bootsma, 1993). An older (Pride 5) and two more recent maize hybrids (Pioneer 3902 and Pioneer 3893) were seeded at three plant densities (1, 3.5 and 12 plants m^{-2}, hereafter referred to as D1, D2 and D3, respectively). Planting dates were 13 May 1999, 26 May 2000 and 9 May 2001. It was assumed that the three hybrids used are representative of breeding efforts in different eras, i.e. Pride 5 in the 1950s, Pioneer 3902 in the 1980s, and Pioneer 3893 in the 1990s. All seeds were seeded with handplanters at two seeds per hill and thinned to one seedling per hill about three weeks after planting. Prior to seeding, 600 kg ha^{-1} 20-20-10 fertilizer and 3L ha^{-1} of atrazine (2-chloro-4-ethilamino-6-isopropylamino-S-triazine) were applied to the soil. Weed control was completed by applying 0.28 kg ha^{-1} bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) before leaf number six was fully expanded and by manual weeding. When the previous crop was maize (i.e., 2001), soil insects were controlled by applying 10 kg ha^{-1} Force (Tefluthrin 1.5 g) which was incorporated into the soil one day before seeding.

The experimental layout was a split plot design with the main plots (plant density) arranged in a randomized complete block and replicated four times. Hybrids were assigned to subplots. Each subplot was 54.7 m^2 (six 0.76 m wide rows x 12 m long) and two additional rows were used to separate plots of low and high plant density treatments. Two sample areas of 4.56 m^2 were marked in each subplot for future harvests. The sample area consisted of two 3-m long center rows, separated on each side by a 2-m border.

Data collection and measurements

Crop development was monitored weekly on 10 tagged plants per subplot. Silking (i.e., the time when the first silks emerged from the husks) was recorded when 50% of tagged plants showed that development stage. At silking, plants from a 4.56 m^2 sample area were cut at ground level and divided into sample and sub-sample portions. The sub-sample consisted of either five (D1 and D2) or 10 (D3) plants selected randomly from the plants in the sample. The sub-sample was separated into leaves, stem plus leaf sheaths, tassels and ears (ears and shanks). After the fresh weights of the sample and sub-sample were recorded, moisture content of the sub-sample was determined by drying the sub-sample at 80°C until weight did not change for two consecutive weighing dates. Total weight of the sample area was estimated by multiplying total sample fresh weight and dry matter percentage of sub-sample. After physiological maturity, plants from a 4.56 m^2 sample were separated into stover and ear portions, dried at 80°C and weighed. Ears were counted and threshed to determine grain yield (0% grain moisture).

Green leaf area of individual leaves of five plants in the sub-sample taken at silking was measured with a LI-3000 leaf area meter (LI-COR, Lincoln, Nebraska) and leaf area index (LAI) was calculated by multiplying mean leaf area per plant by plant density and dividing by area of the sampled portion. Starting at approximately one week after silking, the fraction of each leaf that was green was visually rated at 10-day intervals for each of 10 tagged plants per plot. The percentage of leaf senescence for a specific leaf was calculated by dividing the green area by its leaf area measured at silking, subtracting from one and multiplying by 100. The decline in green leaf area index was estimated by applying this procedure to total leaf area per plant. Relative leaf area index (RLAI) after silking was calculated as the ratio between LAI of green leaf area and LAI at silking. Dry matter mobilization during the grain-filling period was calculated as changes in stover weight between silking and maturity. Change in stover weight is an indication of source:sink ratio (Tollenaar and Daynard, 1982). Relative rate of senescence (RRLS) was calculated as the coefficient of a linear regression model describing the relationship between RLAI and days after silking (DAS).

Data analysis

All data were analyzed by using procedures included in the SAS package (SAS, 1997). Data from three years were combined and analyzed by using the PROC MIXED procedure. Both density and hybrid were assumed as fixed effects and year as random effect. Analysis of variance for each individual year was executed on transformed data (Fernandez, 1992) using PROC GLM procedure. When the analysis of variance indicated the presence of significant differences, simple mean comparisons were made with the LSD test. Regression coefficients were obtained by the PROC REG procedure.

RESULTS

Weather Conditions

Weather conditions differed markedly among three growing seasons (Table 2.1). The 1999 growing season was characterized by high temperature and high levels of incident solar radiation and adequate rainfall. In contrast, the 2000 growing season was characterized by low temperature during vegetative stages, low incident solar radiation and an above-average precipitation. In 2001, the growing season was characterized by high temperature and solar radiation and dry conditions around silking (i.e., July). During this growing season, dry conditions around silking were followed by rainfalls during the grain-filling period. The mean grain yield in the experiment varied with growing season as 1999>2001>2000. Due to this annual variation, the year effect was significant for various variables in the combined analysis.

LAI at silking

Green leaf area index at silking differed between densities and hybrids responded differentially to changes in plant density in one out three growing seasons. LAI for all three plant densities tended to be lower in Pride 5 than Pioneer 3902 and Pioneer 3893. This general pattern was observed during the three years of the experiment (Table 2.2). Analysis of variance indicated a significant year and year X hybrid effect (Appendix 1). In 1999, LAI in more recent hybrids was 7, 20 and 25% greater than that of Pride 5 for D1, D2 and D3, respectively. These differences were 30, 21 and 20% for the 2000 growing season and 28, 17 and 14% for the 2001 growing season. In general, LAI differences between the two newer hybrids were smaller.

Table 2.1. Mean temperature, incident solar radiation, and rainfall at the Elora Research Station during the growing season in 1999, 2000 and 2001.

		May	June	July	August	September
Mean temperature (° C)	1999	14.2	18.4	20.9	17.5	14.8
	2000	12.7	17.5	18.1	18	13.1
	2001	13.5	18.0	18.4	20.1	14.1
Incident solar radiation ($MJ\ m^{-2}\ d^{-1}$)	1999	12.6	21.2	21.2	16.6	13.2
	2000	4.2	19.2	20.0	17.2	12.6
	2001	19.8	20.6	21.8	19.0	14.4
Rainfall (mm)	1999	45	131	64	56	109
	2000	130	195	107	61	72
	2001	71	63	44	78	87

In spite of the magnitude of changes in leaf area, the vertical distribution of green leaf area at silking across the canopy was rather stable across plant densities and years. The central section of the canopy represented around 50% in all three hybrids, regardless of plant density or year. However, hybrids differed in the proportion of leaf area represented by the top and bottom section of the canopy. The proportion of canopy represented by the top section was higher in Pride 5 than Pioneer 3902 and Pioneer 3893, and the opposite was the case for the bottom section of the canopy (Table 2.3).

Table 2.2. Leaf area index (LAI) at silking of three maize hybrids grown at three plant densities in 1999, 2000 and 2001.

Plant density	Hybrid	Year		
		1999	2000	2001
plant m^{-2}		LAI (m^2 m^{-2})		
1	Pride 5	0.51	0.39	0.39
	Pioneer 3902	0.54	0.54	0.50
	Pioneer 3893	0.55	0.48	0.50
	LSD (0.05) †	0.05	0.03	0.04
3.5	Pride 5	1.59	1.50	1.47
	Pioneer 3902	1.87	1.85	1.73
	Pioneer 3893	1.96	1.78	1.71
	LSD (0.05)	0.10	0.12	0.16
12	Pride 5	4.23	4.72	4.39
	Pioneer 3902	4.96	5.76	4.93
	Pioneer 3893	5.57	5.60	5.12
	LSD (0.05)	0.48	0.62	0.16
		P-value		
		1999	2000	2001
Plant density (D)		0.0001	0.0001	0.0001
Maize hybrid (H)		0.0001	0.0001	0.0003
D x H		0.0215	0.2403	0.3273

†: least significant difference to compare hybrids within plant densities.

‡: Analysis of variance was performed on transformed data (natural logarithm).

Table 2.3. Vertical leaf area distribution (as percent of total) at silking in 4-leaf intervals. Values represent means across plant densities and years.

Hybrid	Section of canopy†		
	Top	Middle	Bottom
	%		
Pride 5	35.1a‡	48.8 a	16.1 a
Pioneer 3902	25.6 b	48.1 a	26.2 b
Pioneer 3893	24.7 b	47.6 a	27.95 b

† leaf area for each section is the sum of four leaves areas positioned on top, middle or bottom of the canopy.

‡ numbers followed by the same letter within each column are not significantly different according to LSD (0.05).

Rate of leaf senescence

The progress of leaf senescence during the grain-filling period was estimated as the daily reduction in the relative leaf area index from silking to maturity. The relative rate of leaf senescence (RRLS) was the regression coefficient of the linear relationship between relative leaf area index and days after silking. Regardless of plant densities and hybrids, leaf senescence started to accelerate approximately between 25 to 35 days after silking (depending on the growing season), which roughly coincided with the center of the grain-filling period (Figs. 2.1, 2.2 and 2.3). The mean rate of leaf senescence during the second half of the grain-filling period was 7 (1999), 2 (2000), and 5 (2001) times greater than that during the first half of the grain-filling period. When plant densities and hybrids were compared based on the RRLS during the first and second half of the grain-filling period (hereafter referred as P1 and P2), the analysis of variance for each individual growing season indicated that the density X hybrid interaction was not significant for P1 and significant for P2 (Tables 2.4, 2.5 and 2.6).

Rates of leaf senescence during the first half of the grain-filling period were greater in the old than in more recent hybrids. Indeed, RRLS in P1 was two to five times greater in Pride 5 than those observed in Pioneer 3902 and Pioneer 3893 (Tables 2.4, 2.5, and 2.6). Differences between older and newer hybrids were greater in the 1999 and 2000 growing seasons. In 2001, differences between older and new hybrids were much smaller, possibly due to the effect of extremely low rainfall during vegetative stages and drought stress around silking time, which resulted in early senescence of lower leaves in all hybrids. When averaged across hybrids, RRLS during the first half of the grain-filling period in D3 was 80% greater than the mean of D1 and D2 in the 1999 and 2000 growing seasons and RRLS was more than 3 times greater in D3 than in D1 and D2 in 2001.

Rates of leaf senescence during the second half of the grain-filling period were greater in the older than in newer hybrids for all densities used in this study. When averaged across plant densities, differences in RRLS during P2 ranged between 45 and 188% depending on growing season (Tables 2.4, 2.5, and 2.6). Older and more recent hybrids responded differentially to plant density during the second half of the grain-filling period in terms of RRLS during P2, which declined with an increase in plant density in the older hybrid, and had the lowest values at D2 in newer hybrids.

The proportion of LAI at silking that remained green at the end of the growing season ranged from 0 to 32% in the older hybrid and from 18 to 87% in more recent hybrids, depending on plant densities and growing season (Figs. 2.1, 2.2 and 2.3).

Table 2.4. Relative decline in leaf area index (LAI) during the grain filling period of three maize hybrids grown at three plant densities in 1999.

Plant density	Hybrid	Relative decline in LAI †	
		Period 1 ‡	Period 2
		--------% d^{-1}--------	
1 plant m^{-2}	Pride 5	0.59	2.93
	Pioneer 3902	0.16	1.47
	Pioneer 3893	0.12	1.43
3.5 plant m^{-2}	Pride 5	0.29	2.38
	Pioneer 3902	0.03	0.51
	Pioneer 3893	0.03	1.29
12 plant m^{-2}	Pride 5	0.66	2.33
	Pioneer 3902	0.27	2.79
	Pioneer 3893	0.19	3.07
		-------- P-value --------	
Plant density (D)		0.0005 §	0.0001
Maize hybrid (H)		0.0001	0.0002
D x H		0.9553	0.0001

† Relative decline in LAI is the regression coefficient of the relationship between relative leaf area index and days after silking. Values express the percent of reduction per day. The regression was performed once for each hybrid and density in each of four reps, and the calculated coefficients were examined for density and hybrid effects via analysis of variance.

‡ Period 1 and 2 represent approximately the first and second half of the grain-filling period, respectively.

§ Analysis of variance was performed on transformed data (root square).

Table 2.5. Relative decline in leaf area index (LAI) during the grain filling period of three maize hybrids grown at three plant densities in 2000.

		Relative decline in LAI †	
Plant density	Hybrid	Period 1 ‡	Period 2
		% d^{-1}	
1 plant m^{-2}	Pride 5	1.22	3.33
	Pioneer 3902	0.12	1.32
	Pioneer 3893	0.14	1.15
3.5 plant m^{-2}	Pride 5	1.22	2.83
	Pioneer 3902	0.13	0.57
	Pioneer 3893	0.11	0.79
12 plant m^{-2}	Pride 5	1.59	1.88
	Pioneer 3902	0.62	0.69
	Pioneer 3893	0.43	1.07
		P-value	
Plant density (D)		0.0001 §	0.0001
Maize hybrid (H)		0.0004	0.0113
D x H		0.1764	0.0243

† Relative decline in LAI is the regression coefficient of the relationship between relative leaf area index and days after silking. Values express the percent of reduction per day. The regression was performed once for each hybrid and density in each of four reps, and the calculated coefficients were examined for density and hybrid effects via analysis of variance.

‡ Period 1 and 2 represent approximately the first and second half of the grain-filling period, respectively.

§ Analysis of variance was performed on transformed data (root square).

Table 2.6. Relative decline in leaf area index (LAI) during the grain filling period of three maize hybrids grown at three plant densities in 2001.

		Relative decline in LAI †	
Plant density	Hybrid	Period 1 ‡	Period 2
		------------------% d^{-1}	---------------------
1 plant m^{-2}	Pride 5	0.33	4.25
	Pioneer 3902	0.11	1.97
	Pioneer 3893	0.17	1.42
3.5 plant m^{-2}	Pride 5	0.38	3.64
	Pioneer 3902	0.14	1.35
	Pioneer 3893	0.17	1.27
12 plant m^{-2}	Pride 5	1.20	2.12
	Pioneer 3902	0.75	1.51
	Pioneer 3893	0.71	1.34
		----------------- P-value	----------------
Plant density (D)		0.0001 §	0.0325
Maize hybrid (H)		0.0001	0.0001
D x H		0.7630	0.0589

† Relative decline in LAI is the regression coefficient of the relationship between relative leaf area index and days after silking. Values express the percent of reduction per day. The regression was performed once for each hybrid and density in each of four reps, and the calculated coefficients were examined for density and hybrid effects via analysis of variance.

‡ Period 1 and 2 represent approximately the first and second half of the grain-filling period, respectively.

§ Analysis of variance was performed on transformed data (root square).

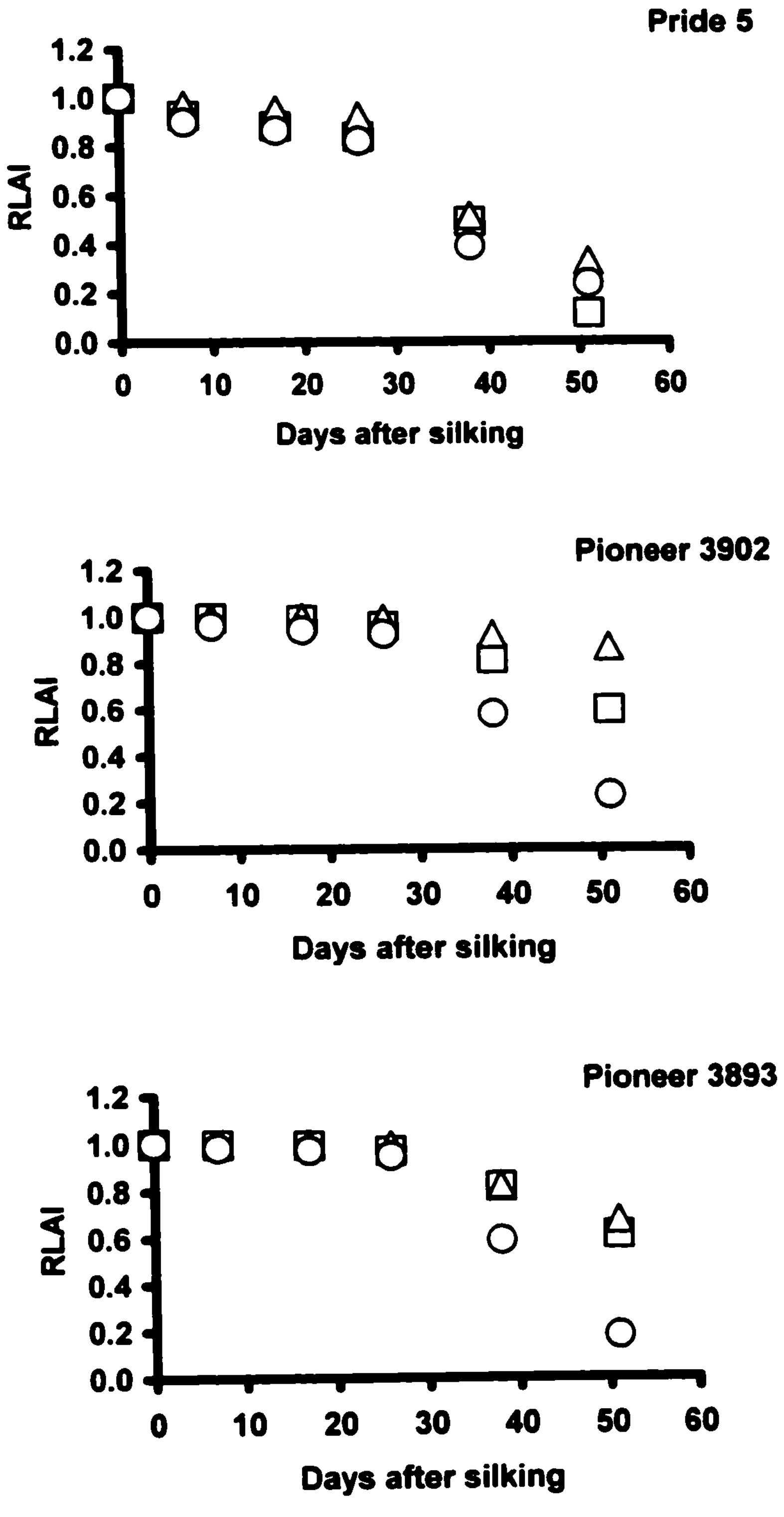

Fig. 2.1. Leaf area index relative to silking (RLAI) for three maize hybrids (Pride 5, Pioneer 3902, and Pioneer 3893) grown at 1 (squares), 3.5 (triangles), and 12 (circles) plants m^{-2} in 1999.

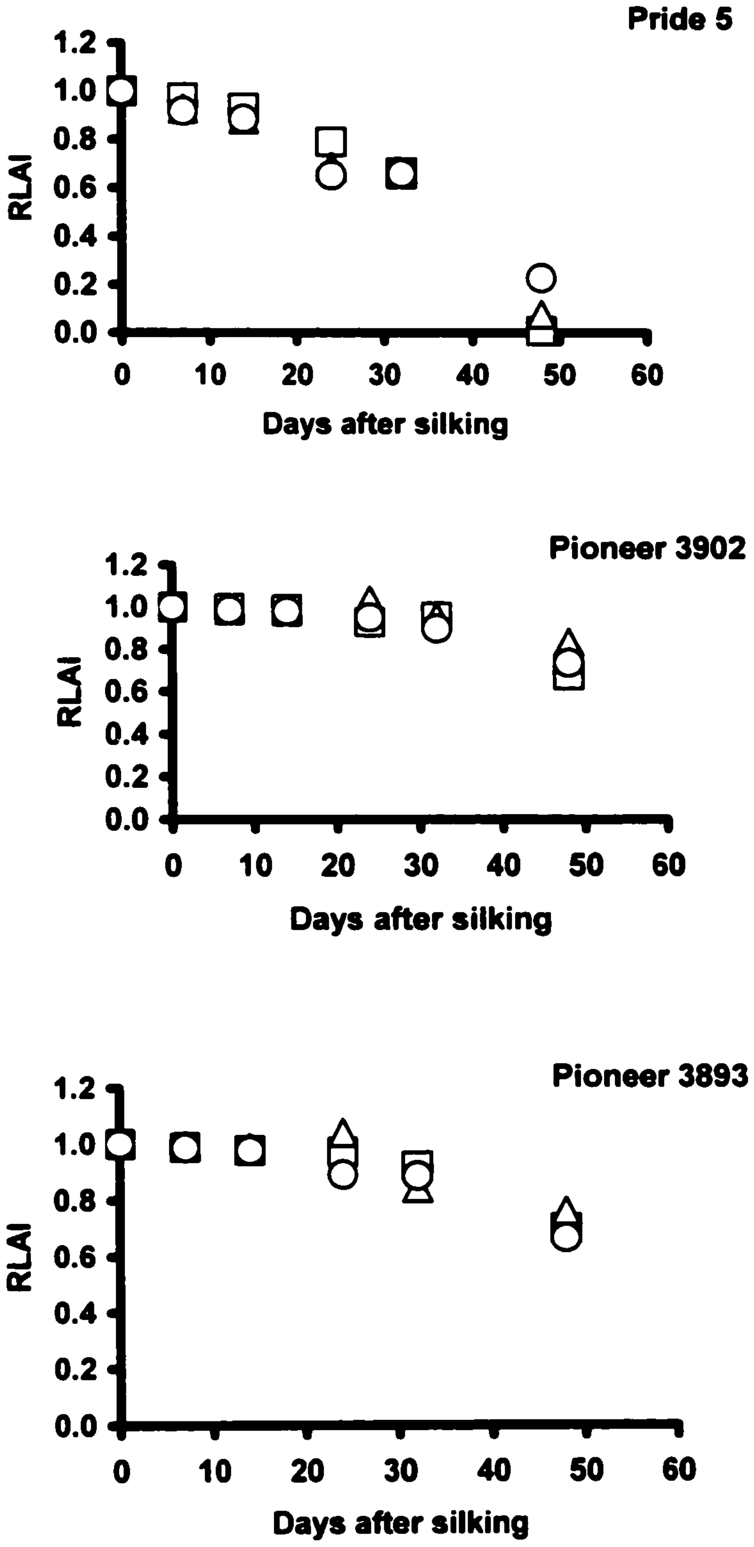

Fig. 2.2. Leaf area index relative to silking (RLAI) for three maize hybrids (Pride 5, Pioneer 3902, and Pioneer 3893) grown at 1 (squares), 3.5 (triangles), and 12 (circles) plants m^{-2} in 2000.

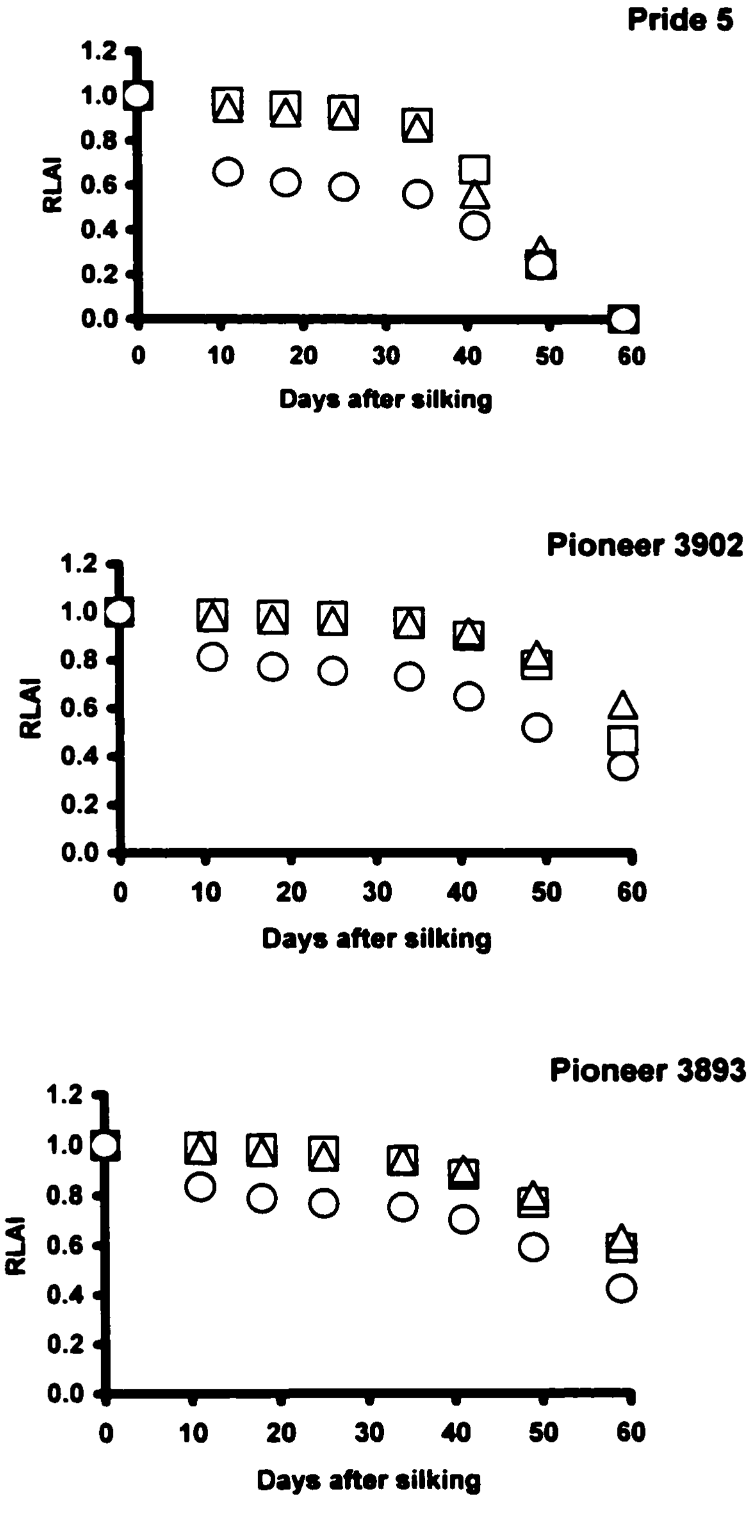

Fig. 2.3. Leaf area index relative to silking (RLAI) for three maize hybrids (Pride 5, Pioneer 3902, and Pioneer 3893) grown at 1 (squares), 3.5 (triangles), and 12 (circles) plants m^{-2} in 2001.

Profile of visual leaf senescence

A top-bottom senescence profile was detected in two out three growing seasons. In this profile, initial visual symptoms of senescence were observed in the oldest leaves and progressed upward the plant, but later, as the grain-filling period advanced, the topmost leaves started to senesce and senescence progressed downward the plant. Therefore, a simultaneous progression of senescence downward and upward ended with top and bottom leaves having a greater percent of senescence than central leaves. The top-bottom profile of leaf senescence was observed as early as 40 days after silking (data not shown). The presence of this profile was influenced by the growing season and its magnitude was altered by both hybrid and plant density. For instance, the profile was observed in 1999 and 2001, but not in 2000 (Figs. 2.4, 2.5, and 2.6), it was more marked in more recent hybrids grown at D1 and D2 during 1999 (Fig. 2.4B, C, E, F) and it was apparent in all densities during 2001 (Fig. 2.6). For Pride 5, the top-bottom leaf senescence profile was less evident than in other hybrids and top leaf senescence was restricted to the topmost leaf in the 2001 growing season (Fig. 2.6). Leaf senescence progressed from the bottom and top leaf did not start to senesce until as late as 48 day after silking in all treatment during 2000 (Fig. 2.5).

Changes in stover weight

Changes in stover weight between silking and maturity are indicative of the source:sink ratio. Changes in stover weight varied with plant density and hybrid and the magnitude of those changes varied with growing season (Table 2.7). It was anticipated that stover weight between silking and maturity would increase in the lowest plant density (D1), because of a relatively high source:sink ratio, and decrease in the highest plant density (D3), because of a relatively low source:sink ratio (Rajcan and Tollenaar, 1999). This pattern was

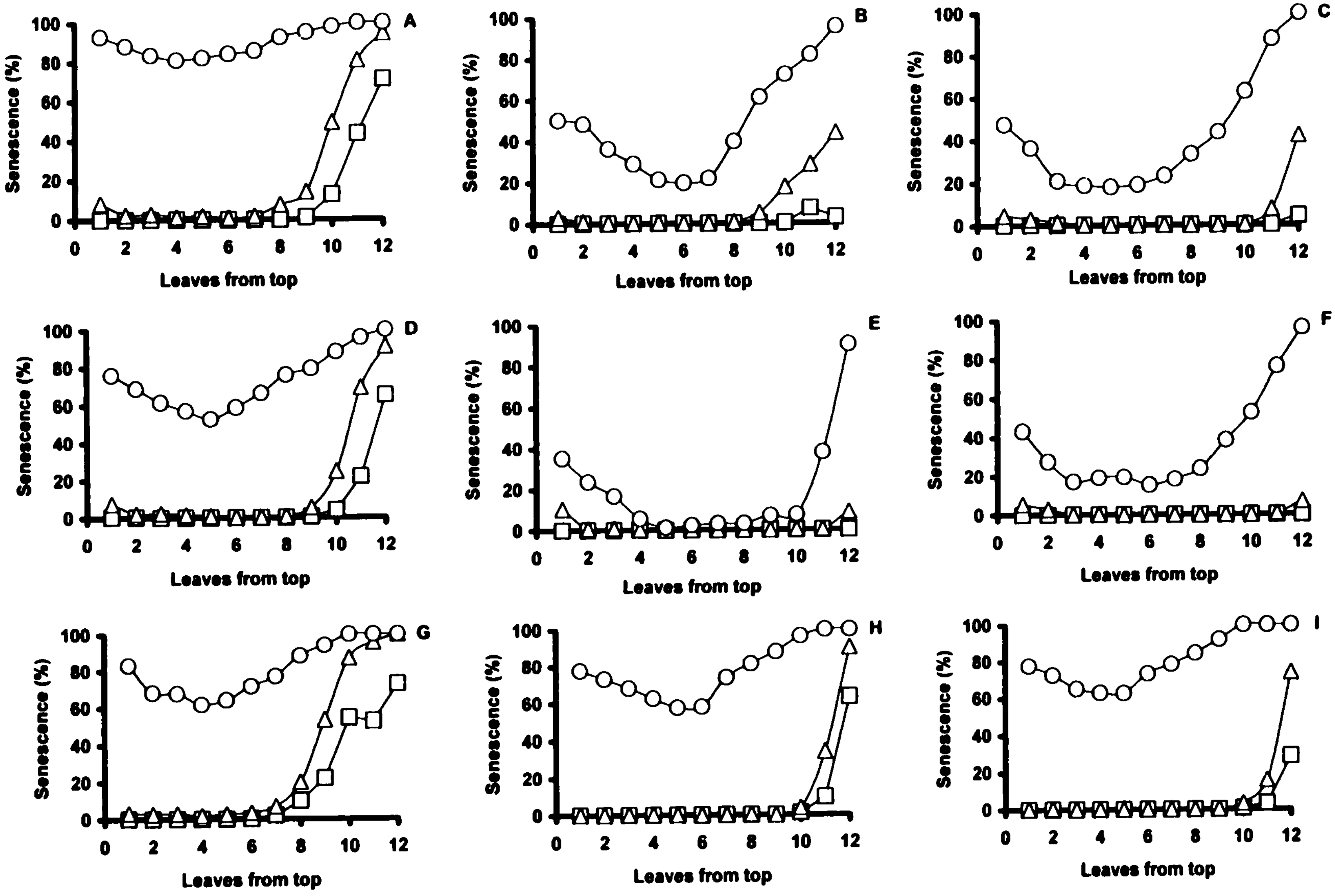

Fig. 2.4. Percentage of senescence as related to nodal position at 7 (squares), 26 (triangles), and 51 (circles) days after silking in Pride 5 (A, D, G), Pioneer 3902 (B, E, H) and Pioneer 3893 (C, F, I) grown at three plant densities in 1999: 1 plant m^{-2} (A, B, C), 3.5 plant m^{-2} (D, E, F), and 12 plant m^{-2} (G, H, I). LSD (0.05) to compare leaves within each plant density is 15.9.

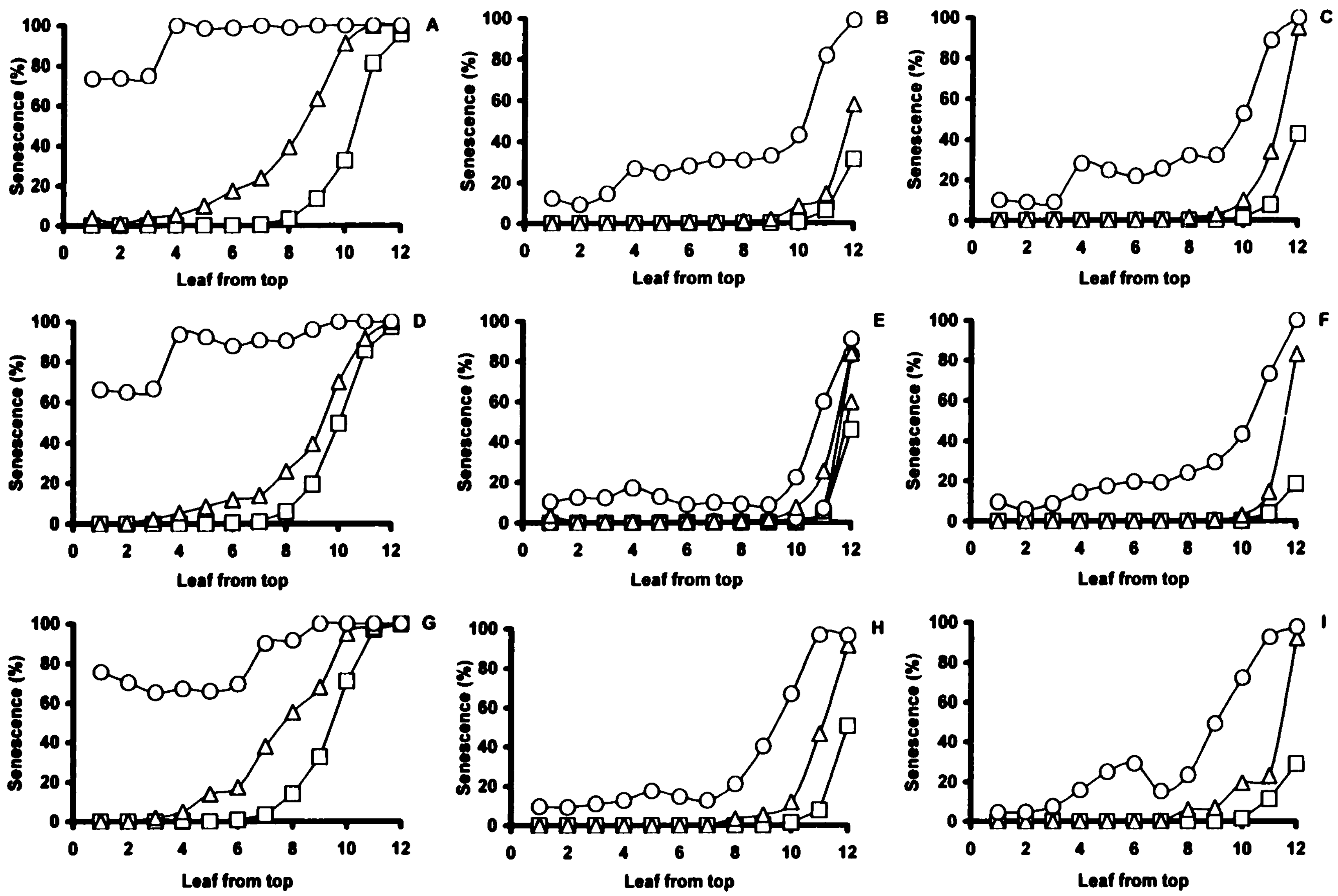

Fig. 2.5. Percentage of senescence as related to nodal position at 7 (squares), 24 (triangles), and 48 (circles) days after silking in Pride 5 (A, D, G), Pioneer 3902 (B, E, H) and Pioneer 3893 (C, F, I) grown at three plant densities in 2000: 1 plant m^{-2} (A, B, C), 3.5 plant m^{-2} (D, E, F), and 12 plant m^{-2} (G, H, I). LSD (0.05) to compare leaves within each plant density is 11.4.

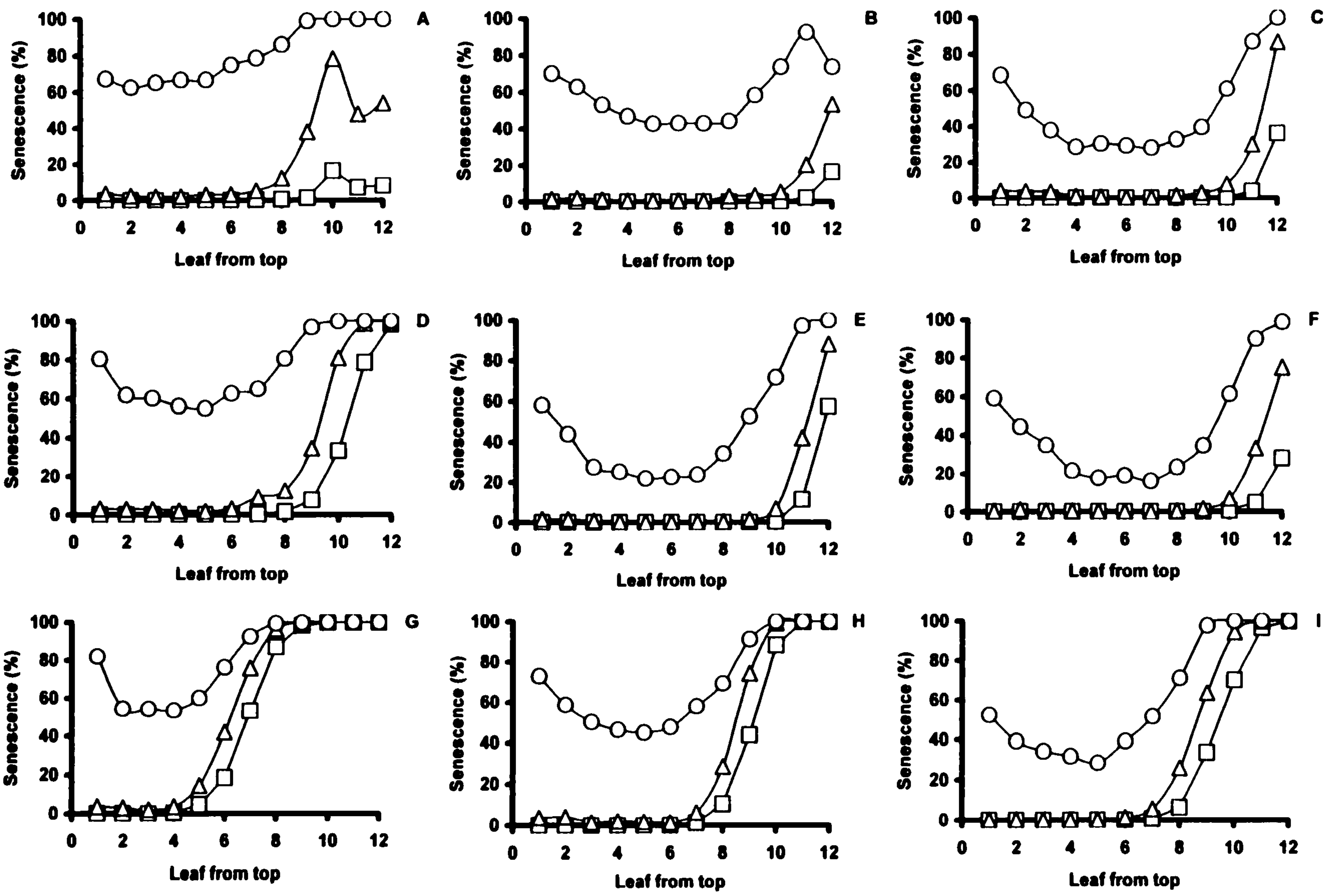

Fig. 2.6. Percentage of senescence as related to nodal position at 11 (squares), 34 (triangles), and 59 (circles) days after silking in Pride 5 (A, D, G), Pioneer 3902 (B, E, H) and Pioneer 3893 (C, F, I) grown at three plant densities in 2001: 1 plant m^{-2} (A, B, C), 3.5 plant m^{-2} (D, E, F), and 12 plant m^{-2} (G, H, I).
LSD (0.05) to compare leaves within each plant density is 16.9.

observed in more recent hybrids, where stover weight between silking and maturity increased up to 54% in D1 and decreased up to 17% in D3. In contrast, stover weight of Pride 5 decreased at both plant densities. In general, the two groups of hybrids had comparable variations in stover weight when grown at D2 in 1999 and 2000, but in 2001, stover weight of Pioneer 3902 and Pioneer 3893 increased two times more than in Pride 5.

Grain Yield

Grain yield varied with plant density and hybrid for each of the three growing seasons (Table 2.8). Hybrids differed in their response to plant density during 2000 and 2001 growing seasons as shown by the significant density X hybrid interaction. Grain yield in Pride 5 was consistently lower than Pioneer 3902 and Pioneer 3893 across all plant densities. The only exception occurred during 2001 growing season in D2, when grain yields of all three hybrids were comparable. Grain yield in the most recent hybrid (Pioneer 3893) tended to be greater than Pioneer 3902 and, when averaged across growing seasons, differences between these two hybrids were greater in D3 (66%).

Rate of leaf senescence, changes in stover weight, and yield

For all three growing seasons, the rate of leaf senescence during the second half the grain-filling period was inversely correlated ($P < 0.05$) with changes in stover weight, indicating that remobilization of dry matter from the stover to the ear occurred concomitantly with accelerated leaf senescence (Table 2.9).

Rate of leaf senescence during P2 was also negatively associated ($P < 0.05$) with grain yield in 2000 and 2001 growing seasons, accounting for approximately one third of grain yield variation in each year (Fig. 2.7). The slope of this relationship was greater in 2000 than 2001, i.e., during the second half of the grain-filling period grain yield declined 139 kg ha^{-1} (2000) and 79 kg ha^{-1} (2001) per each percent of increase in the rate of leaf senescence. In contrast, the rate of leaf senescence during P2 was not significantly associated with grain yield ($P > 0.05$) in 1999, the growing season with the highest yield. Changes in stover weight during the grain-filling period were not associated with grain yield in any of three growing seasons (Table 2.9).

Table 2.7. Relative changes in stover weight from silking to maturity in three maize hybrids grown at three plant densities in 1999, 2000 and 2001.

Plant density plant m^{-2}	Hybrid	1999	2000	2001	Mean
		Relative change in stover weight (%)			
1	Pride 5	-9 a †	1 b	-12 b	-7
	Pioneer 3902	28 a	48 a	41 a	39
	Pioneer 3893	25 a	54 a	54 a	44
	Mean	14	34	28	
3.5	Pride 5	3 a	-8 a	30 b	8
	Pioneer 3902	10 a	8 a	70 a	29
	Pioneer 3893	-1 a	16 a	75 a	30
	Mean	4	5	58	
12	Pride 5	-25 b	-1 a	-20 b	-15
	Pioneer 3902	-4 a	-6 a	6 a	-1
	Pioneer 3893	-17 b	-1 a	0 ab	6
	Mean	-15	-3	-5	
		P-value			
Plant density (D)		0.0262‡	0.0044	0.0024	
Maize hybrid (H)		0.0097	0.0001	0.0001	
D x H		0.1840	0.0257	0.1501	

†: Values within plant density followed by the same letter are not significant different (P > 0.05).

‡ Analysis of variance was performed on transformed data (value + 100).

Table 2.8. Grain yield of three maize hybrids grown at three plant densities in 1999, 2000 and 2001.

Plant density	Hybrid	Year		
		1999	2000	2001
plant m^{-2}		Grain yield (g m^{-2})		
1	Pride 5	207	89	192
	Pioneer 3902	374	174	293
	Pioneer 3893	523	178	350
	LSD (0.05) †	121	40	22
3.5	Pride 5	472	159	518
	Pioneer 3902	685	385	566
	Pioneer 3893	862	443	554
	LSD (0.05)	188	130	61
12	Pride 5	536	178	276
	Pioneer 3902	850	637	430
	Pioneer 3893	924	740	713
	LSD (0.05)	114	203	177
		P-value		
Plant density (D)		0.0001	0.0001	0.0001
Maize hybrid (H)		0.0001	0.0001	0.0008
D x H		0.2549	0.0030	0.0001

†: least significant difference to compare hybrids within plant densities.

Table 2.9. Correlation coefficients between relative rate of leaf senescence (RRLS) during the second half of grain filling period, changes in stover weight (▲STW), i.e., change in dry weight of leaves, stems, husks, and cobs from silking to maturity, and grain yield in 1999, 2000, and 2001. For each year data included three hybrids and three densities (*n*=36).

Variable	▲STW			Yield		
	------------------	------------------	------------------ *r*	------------------	------------------	------------------
	1999	2000	2001	1999	2000	2001
RRLS †	-0.52*	-0.28*	-0.33*	NS	-0.58*	-0.56*
▲STW	-	-	-	NS	NS	NS

* significant at 0.05

† Relative rate of leaf senescence (RRLS) is the regression coefficient of the relationship between relative leaf area index and days after silking. Values express the percent of reduction per day. The regression was performed once for each hybrid and density in each of four reps, and the calculated coefficients were examined for density and hybrid effects via analysis of variance.

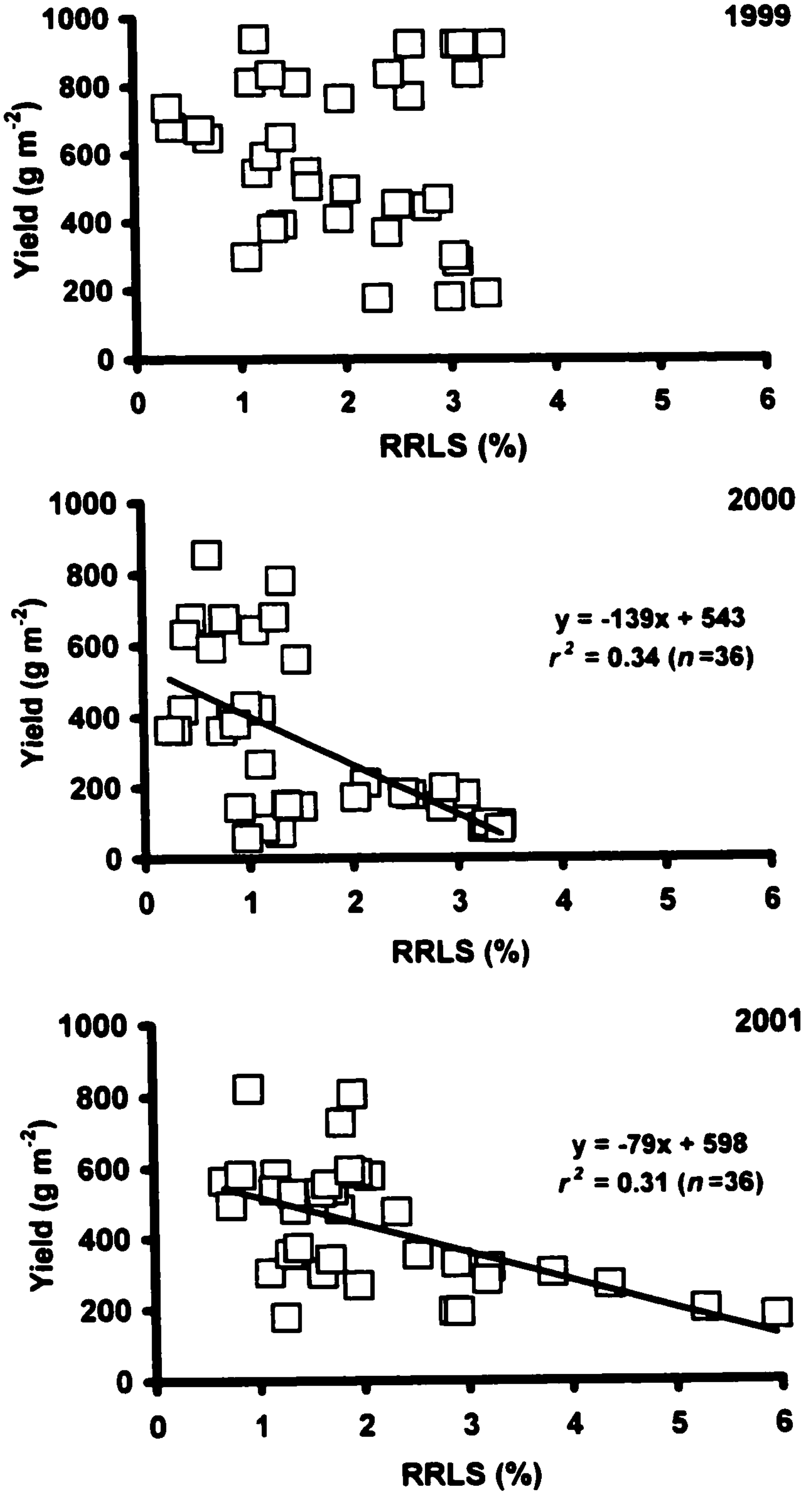

Fig. 2.7. Relationship between grain yield and relative rate of leaf senescence (RRLS) during the second half of the grain filling period in three years. For each year data include three hybrids and three densities.

DISCUSSION

The objective of the present work was to quantify leaf senescence in terms of its visual symptoms in an older and two more recent maize hybrids during the grain-filling period. Results support the contention that the improved performance during the grain-filling period in newer vs. older hybrids is associated with a greater green leaf area index. A greater green leaf area during the grain-filling period in new hybrids was the result of greater green LAI at silking, delayed onset of leaf senescence, and lower rates of leaf senescence.

An increase of leaf area per plant in newer relative to older hybrids had been identified as one of the physiological traits contributing to grain yield improvement in short growing-season environments (Tollenaar and Daynard, 1978). Our results showed that green LAI at silking was consistently greater in newer than in the older hybrid across a wide range of plant densities used in this study. When averaged across all three growing seasons, maximum green LAI was around 20% greater in Pioneer 3902 and Pioneer 3893 than in Pride 5. The hybrid X plant density interaction was significant in one out of three growing seasons, as has been previously reported by Tollenaar (1991). This interaction may be attributable to a similar green LAI for all the three hybrids in D1 during 1999, an apparently favorable growing season that showed the greatest grain yield and, presumably, the lowest stress conditions. Under less favorable growing seasons (i.e., the 2000 and 2001 growing seasons), the older and newer hybrids in D1 significantly differed in green LAI at silking, and this was also observed in D2 and D3.

Although LAI differed markedly among plant densities at silking, the vertical distribution of leaf area across the canopy was rather stable for each group of hybrids.

Approximately one half of total green leaf area was positioned in the central section of the canopy for all three hybrids, a result that agree with Tollenaar and Daynard (1978) observations. Differences between the older and newer hybrids were found in both the top and bottom sections of the canopy: the older hybrid had a greater proportion of green leaf area in the top section and lower proportion in the bottom section. These small but significant changes in the vertical leaf area distribution reflect a plant architecture closer to a Christmas-tree shape in more recent hybrids, a trait thought be more favorable for light interception (Troyer, 2000). A relatively smaller leaf area in the top section results in greater penetration of light to the ear leaf (Dwyer et al., 1992), in less mutual shading and, possibly a delay in senescence of bottom leaves as plant density increases. Troyer and Rosembrook (1983) speculated that the Christmas-tree shape vertical distribution of leaf area might be a consequence of selection under high plant density, a key component in the apparent increase in stress tolerance in modern temperate maize germplasm (Tollenaar and Wu, 1999). A greater leaf area above the ear has been also associated with lower optimum plant density for grain yield (Dwyer et al., 1992).

The maintenance of a green leaf area during reproductive stages is of particular importance to sustain high grain yield under acute or sub-lethal stress (Nooden, 1988). Imposing higher plant densities constitutes a stress condition that is reflected in a decreased yield per plant (Tollenar and Wu, 1999; Andrade et al., 1999) and accelerated leaf senescence may be one cause of this reduction. In line with this concept, maize yield improvement over the years has been related, at least partly, with an improved tolerance to abiotic stress. Our results show that more recent hybrids had a delayed onset of leaf senescence and lower rates of senescence during the first half of the grain-filling period than the older hybrid. We identified a period between silking and approximately the mid-point of the grain-filling period, when leaf senescence was apparent in Pride 5 but not in more recent hybrids. The reduction in green leaf area relative to the leaf area at silking at this time ranged between 12 and 44% in the older hybrid and was almost negligible for the newer hybrids. Since these reductions of LAI occurred regardless of plant density (i.e., density X hybrid interaction was not significant), its possible impact on grain yield of maize grown at commercial densities can be estimated. For instance, in Pride 5 grown at a commercial plant density resulting in a LAI=3 and a light extinction coefficient of the canopy k=0.65, a 44% reduction in LAI would result in a reduction in light interception from 86 to 66% of incident solar radiation.

The detrimental impact of reductions in green leaf area and its consequent decline of assimilatory capacity during the grain-filling period on kernel number and kernel weight have been previously reported (Tollenaar and Daynard, 1978, Uhart and Andrade, 1995). Maize grain yield depends on dry matter accumulation around silking for kernel set and on dry matter accumulation during the grain-filling period for attaining a high kernel weight (Andrade et al., 1999; Tollenaar, 1991). Grain yield differences between older and newer hybrids have been associated with an enhanced crop growth rate around silking and during the grain-filling period (Tollenaar et al., 1992; Tollenaar and Aguilera, 1992) and those differences become greater under stressful conditions (Tollenaar and Wu, 1999). In the experiment reported herein, leaf senescence in Pride 5 observed immediately after silking would be associated with an early and limited flux of current assimilates and, consequently, with a low capacity to set kernels. This is in contrast with results reported by Crosbie (1982) for Corn Belt hybrids, which showed that differences in leaf senescence between older and more recent hybrids became apparent only after 3 wk after silking. In fact, competition for assimilates among kernels take place during the whole grain-filling period (Borras and Otegui, 2001).

As has been reported previously (Rajcan and Tollenaar, 1999), rate of leaf senescence in both older and newer maize hybrids increased during the second half of the grain-filling period and rates of leaf senescence were greater at the high plant density. Tollenaar (1992) reported that leaf senescence was accelerated at high plant densities during advanced reproductive stages. A significant density X hybrid interaction for rate of leaf senescence in P2 but not in P1 indicates that a differential response to plant density in terms of visual leaf senescence occurred among hybrids late in the growing season which could be associated with the ability to sustain grain growth. Differential progress in leaf senescence during the second half of the grain-filling period may be associated with changes in source:sink ratio. In this study, the rate of leaf senescence during the second half of the grain-filling period was associated with a decrease in source:sink ratio, which was reflected by a reduction in stover weight between silking and maturity (Table 2.9). This reduction was greater in D3, a plant density characterized by a low source:sink ratio, and can be attributed to disproportional increases in kernel number as the number of plants per unit area increases. Indeed, grain yield, which is mainly determined by kernel number and consequently an indicator of sink size, increased more than the leaf area, which is an indicator of source size, when plant density increased from D2 to D3 in all three hybrids. However, the increase in grain yield was 60% greater in the newer hybrids than in Pride 5, while the concomitant increase in green LAI at silking was 20%. If green leaf area at silking and grain yield are assumed as indicators of source and sink size, respectively, more recent hybrids would have a less balanced source:sink ratio than that observed in the older hybrid. Moreover, this "unbalanced" source:sink ratio could be exacerbated late in the season as a result of the natural progress in leaf senescence and the growing demand for assimilates and nitrogen as the grain-filling period advances. The combined effect of decreasing source strength while increasing sink strength would account, in part, for the high rate of senescence during the second half of the grain-filling period in newer hybrids (Tollenaar and Daynard, 1982). These results are in agreement with those reported by Rajcan and Tollenaar (1999) who utilized defoliation treatments to distort the source-sink ratio. Therefore, although both the older and newer hybrids showed enhanced rates of leaf senescence during the second half of the grain-filling period at high plant density, the primary reason for an accelerated leaf senescence seems to be different for each groups of hybrids.

Indeed, whereas in the older hybrid the leaf senescence would be accelerated by the remobilization of assimilates (Tollenaar and Daynard, 1982) as a result of an early reduction in green LAI during the first half of the grain-filling period (reduced source), in newer hybrids leaf senescence would be also accelerated by the remobilization of assimilates, but in this case as a result of an enhanced ability to set high kernel number (increased sink) around silking time (Tollenaar et al., 1992). Therefore, setting a large kernel number at the beginning of reproductive stages in newer hybrids would become detrimental in a sense of accelerating leaf senescence during the last part of the growing season. Since any progress in grain yield represents a sink increase, it seems reasonable to speculate that future challenges for maize breeders likely will include plant traits related with delayed leaf senescence in order to match the growing assimilate demand. Thus, an optimum type of plant with bearing traits oriented to match the growing sink size could be conceived.

When the progress of leaf senescence across the canopy leaf was examined, a top-bottom profile of leaf senescence during the second half of the grain-filling period was observed in two out of three growing seasons, and this profile was more distinct in the two newer hybrids than in the older hybrid. In this profile, senescence of leaves positioned in the top section of the canopy occurred earlier than leaves directly below, despite the fact that the upper leaves were younger and the advantageous position of the former for photosynthesis (Tollenaar and Daynard, 1978). The top-bottom profile of leaf senescence was most distinct in high yielding growing seasons. Thus it could be hypothesized that the top-bottom profile of senescence reflects a physiological trait of newer hybrids oriented to maximize grain yield, and would constitute a useful ideotype for leaf senescence and grain yield. The fact that this profile was observed most clearly in the high yielding growing seasons supports this hypothesis.

Grain yield was consistently higher in more recent hybrids and was not associated with changes in stover weight (Table 2.9). Grain yield varied widely among growing seasons and, within each plant density, the magnitude of the differences among hybrids also varied substantially among growing seasons. Our results clearly demonstrate that the grain yield of more recent hybrids is greater than that of the older hybrids across the wide range of plant densities used in this study. The greater grain yield in newer maize hybrids under high plant density support the contention that yield improvement is associated with increased stress tolerance (Tollenaar, 1992). The increase in grain yield from D1 to D2 in Pride 5 was the smallest and Pride 5 was the only hybrid that showed a reduction in grain yield as plant density increased from D2 to D3. Moreover, a comparison of the two more recent hybrids showed that grain yield of Pioneer 3893 (released in the nineties) was consistently greater than that of Pioneer 3902 (released in the eighties) in D3. Grain yield differences between older and newer maize hybrids have been previously reported (Tollenaar, 1989a) and differences have been associated with visual symptoms of leaf senescence during the grain-filling period (Crosbie, 1982; Tollenaar, 1991; Rajcan and Tollenaar, 1999), and *stay green* (Duvick and Cassman, 1999).

CONCLUSIONS

Historically, delaying leaf senescence has been a prime target for crop improvement (Thomas and Howarth, 2000) and it continues to be an avenue for increasing the total amount of carbon fixed by the crop. Results of this study show that differences in grain yield between older and more recent maize hybrids could be accounted for, in part, by a differential progress in visual symptoms of leaf senescence. Our study identified two distinctive periods delimited by a sudden increase in the rate of leaf senescence occurring around the middle of the grain-filling period. During the first period, visual senescence progressed in the older hybrid and was almost negligible in newer hybrids. During the second period, the rate of visual senescence accelerated in both older and more recent hybrids. A top-bottom profile of leaf senescence became apparent during the second period, with leaves in the central section of the canopy being the last leaves to senesce. This profile was evident in growing seasons characterized by more favorable conditions for grain yield and the profile was more distinct in newer hybrids and, possibly, would represent an ideal type of plant for visual senescence and grain yield. The top-bottom profile of leaf senescence also would be in agreement with previous results demonstrating that leaves in the vicinity of ear have greater photosynthesis rate and senesce more slowly than the other leaves (Dwyer and Stewart, 1986a).

CHAPTER III

CHANGES IN FUNCTIONAL SYMPTOMS OF LEAF SENESCENCE ASSOCIATED WITH MAIZE GRAIN YIELD IMPROVEMENT

ABSTRACT

Genetic improvement in grain yield of maize (*Zea mays* L.) has been associated with a delayed appearance of leaf senescence symptoms. The decline in the photosynthetic process constitutes a functional symptom of leaf senescence that would affect the accumulation of dry matter during the grain-filling period of maize (*Zea mays* L.) and, consequently, grain yield. The objectives of this study were i) to examine functional symptoms of leaf senescence in an older (Pride 5) and two more recent maize hybrids (Pioneer 3902 and Pioneer 3893), and ii) to analyze seasonal and diurnal decline in leaf carbon exchange rate (CER) during the grain-filling period. Functional symptoms of leaf senescence (FS), defined as the decline in leaf CER, were examined in plants grown in a hydroponic system established in the field during 1999 to 2001. CER declined as the growing season progressed and the decline was greater in Pride 5 than in Pioneer 3902 and Pioneer 3893. A similar trend was found for leaf conductance and the Fv/Fm ratio. These functional symptoms of senescence occurred in leaves that showed no apparent visual symptoms of senescence. Leaf CER during the day showed an afternoon decline but no difference between hybrids was found in this respect. This study confirms that the better productivity of more recent hybrids during the grain-filling period not only results from a delayed visual senescence but also from a delay in functional senescence. Results also indicate that further advances in maize yield improvement will likely occur through coupling 'stay green' with a high photosynthesis rate maintained through the season rather than simply extending green leaf area duration *per se*.

INTRODUCTION

The maintenance of an active photosynthetic system until physiological maturity is a key to improve agronomic performance of maize (*Zea mays* L.) grown in short-season regions (Tollenaar and Daynard, 1978). Maize hybrids that "stay green" until late in the season have been shown to be more productive than hybrids that show early symptoms of leaf senescence (Hageman and Lambert, 1988), probably due to more sustained photosynthesis during the grain-filling period.

Senescence represents an endogenously controlled degenerative process that leads to death (Leopold, 1975). Senescence of leaves is characterized by a series of biochemical and physiological events that constitute the final phase of leaf development from full expansion until death (Smart et al., 1995). During senescence leaf cells undergo coordinated changes in cell structure, metabolism and gene expression (Nooden, 1988) that, once integrated at the leaf level, are reflected as both visual and functional symptoms of leaf senescence. Loss of chlorophyll is the most obvious and prominent expression of visual senescence (VS) and the rate of degradation of chlorophyll is often considered a measure of the deterioration of the photosynthetic process (Thimann, 1980; Thomas and Stoddart, 1980). Changes in structure and function of chloroplast during leaf senescence (Woolhouse, 1984) may be associated with a decline in photochemical activity of Photosystem II (PSII) and photosynthetic capacity (Grover, 1993; Gan and Amasino, 1995). Consequently, PSII activity and photosynthetic capacity can be considered as a measure of functional leaf senescence (FS).

Visual and functional symptoms of leaf senescence are not always associated. Thomas (1987) found a decline in photosynthesis in leaves of fescue (*Festuca pratensis*) despite retaining a green color, a phenomena referred to as "cosmetic" *stay green* (Thomas and Smart, 1993). In contrast, photosynthesis in leaves of rice (*Oriza sativa* L.) remained high despite a large decrease in chlorophyll content (Murchie et al, 1999). In maize, Connell et al. (1987) and Crafts-Brandner et al. (1984a) found that two *stay green* hybrids differed in photosynthetic rate during the last two to three weeks of the grain-filling period. In addition, Tollenaar and Bruulsema (1988) reported differences in dry matter accumulation after silking in two hybrids with high light absorptance during the grain-filling period. Furthermore, Tollenaar and Aguilera (1992) found differences in dry matter accumulation even when the proportion of light absorptance did not differ between hybrids. Therefore, it may be postulated that some hybrids show rather less photosynthesis than expected by their leaf greenness during the last part of the growing season.

Maize leaves can show lower photosynthesis in the afternoon than could be expected, based on photosynthesis measured in comparable conditions during the morning (Bunce, 1990). This afternoon depression in leaf photosynthesis is a common occurrence (Horton, 2000). It has been reported in rice (*Oryza sativa* L.), soybean (*Glycine max* L.) and maize crops (Murchie et al. 1999; Larson et al., 1981; Bunce, 1990), and has been correlated with grain yield (Black et al., 1995). The nature of this afternoon depression is not well understood, and it has been attributed, among others, to a harmful excess of light (or excitation energy) and stomatal effects (Mohotti and Lawlor, 2001; Baker et al., 1994; Horton, 2000). Moreover, it is not yet clear whether excess excitation energy plays any role in the afternoon depression of senescent leaves with decreased photosynthetic capacity. Murchie et al. (1999) showed that afternoon depression of photosynthesis occurred more in young than old leaves of rice but, to the best of our knowledge, no study of this phenomenon has been reported for maize. Moreover, whether old and newer hybrids differ in their diurnal pattern of photosynthesis has not been investigated.

The objectives of this study were i) to examine functional symptoms of leaf senescence in an old (Pride 5) and two more recent maize hybrids (Pioneer 3902 and Pioneer 3893), and ii) to analyze seasonal and diurnal decline in leaf carbon exchange rate (CER) during the grain-filling period.

MATERIALS AND METHODS

Cultural practices and experimental design

Three short season maize hybrids (Pride 5, Pioneer 3902 and Pioneer 3893) were grown in 25 L pails in a modified hydroponic system established in the field during the 1999, 2000, and 2001 growing season. Details of this system have been previously reported by Ying et al. (2000). In short, nutrient solution was automatically supplied to the pails two or three times per day from planting to physiological maturity. Nutrient solution was composed of 40 g 28-14-14 and 40 g 15-15-30 soluble fertilizers supplemented with trace-mineral salts (Plant Products Co Ltd., Bramalea, Ontario), 20 g NO_3^- NH_4^+, 40 gr $Mg SO_4$ $7H_2O$ and 2 g Mn chelate (12% Mn) dissolved in 100 L of tap water and adjusted with HCl to a pH 5.8. Pails were arranged in eight north-south rows in the field of Cambridge Research Station (43° 39'N, 80° 25' W and 376 m above sea level). Rows were 1.4 m apart and the pails within each row were 0.35 m apart resulting in plant density of 2 plant m^{-2}. Two rows on each side of the experimental area were used as borders.

Approximately 2, 4 and 6 weeks after silking, forecasted sunny days were pre-selected for making measurements. Early in the morning, pails containing five plants of each hybrid were placed 1.5 m apart to allow most leaves to be sunlit (control) and pails containing five plants of each hybrid were place under a frame covered with shade cloth that resulted in a 30% reduction of incident PPFD (shade). All measurements were performed on the second leaf above the primary ear leaf. In an attempt to avoid differences in the structure, biochemical and physiological function from base to tip in a maize leaf, the measurements were taken on a 15-cm long segment of the leaf near its mid-point.

The experimental layout was a factorial with two factors: light level (i.e., control vs. shade) and hybrid. Each individual plant constituted a replication.

Gas exchange, and chlorophyll fluorescence, and chlorophyll content

Carbon exchange rates (CER) and stomatal conductance (COND) were measured using a portable, open flow gas exchange system (LI-6400, LI-COR Inc., Lincoln, NE). Exchange of CO_2 between the leaf sample and the air in the cuvette was measured on 6 cm^2 of leaf area, excluding the midrib. Leaf temperature was set at 28°C and the level of photosynthetic photon flux density (PPFD) inside the chamber was set at 2000 $\mu mol\ m^{-2}s^{-1}$ for the control and 1400 $\mu mol\ m^{-2}s^{-1}$ (70% of the level of the control) for shaded plants. CER measurements were made during the morning (10:00-12:00 eastern daylight saving time, EDST) and afternoon (14:00-16:00, EDST).

Maximum quantum efficiency of PSII was estimated by measuring chlorophyll fluorescence ratio (Fv/Fm) with a Mini-PAM Portable Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich, Germany). For each individual leaf, a series of three consecutive measurements were made. For each day, three (1999), four (2000) and six (2001) measurements were made throughout the day. The first of these was made before sunrise and the last just before sunrise of the subsequent day. Under sunlight conditions, Fv/Fm ratio [(Fm-Fo)/Fm] was determined after 30 min of dark adaptation. Changes in Fm values were used as an indication of processes associated with the protection of PSII, and changes in Fo values were used as an indication of the damage to PSII reaction centers (Maxwell and Johnson, 2000).

Chlorophyll content was estimated by using the SPAD 502 chlorophyll meter (Minolta). Ten readings were made on the same portion of the leaf used for gas exchange and chlorophyll fluorescence determinations.

Data analysis

Data from three years were combined and analyzed by using procedures included in SAS (SAS, 1997). Hybrid was assumed as fixed effects and year as random effect. Data from each individual year were analyzed by using analysis of variance (PROC GLM). Simple mean comparisons were made with protected LSD, only when analysis of variance indicated the presence of differences. The Type I error rate (α) was established at 0.05 for all statistical tests. For chlorophyll fluorescence and SPAD reading, the average of repeated measurements was used to perform the statistical analyses.

RESULTS

Seasonal changes in leaf chlorophyll content, CER, COND and Fv/Fm ratio

In maize plants grown in an outdoor hydroponic system, leaves closer to the main ear remained green longer throughout the grain-filling period than at either at the bottom or the top of the plant. Wind physical damage on the top leaves and aging in the bottom leaves are possible reasons that the most healthy and green section of the canopy during the grain-filling period was the central one. The chlorophyll content of central leaves declined slightly during the grain-filling period. The decline, however, was not significant between 2 and 6 wk after silking (Table 3.1).

The Fv/Fm ratio was close to 0.82 at 2 wk after silking and it tended to decline as the grain-filling period progressed. Although all hybrids showed a significant decline in Fv/Fm ratio at six weeks after silking, the decline was greater in Pride 5 than P-3902 and P-3893 (Table 3.2).

When averaged across three growing seasons, the decline in Fv/Fm ratio was 10% in the old hybrid vs. 6% in P-3902 and 5% in P-3893. Reductions in Fv/Fm ratio were accompanied by changes in minimum (Fo) and maximum fluorescence (Fm). However, the decline was associated more with variations in Fm than Fo (Table 3.3).

Table 3.1. Chlorophyll contents (as measured by SPAD) on the second leaf above the topmost ear of three maize hybrids at three stages of development. Each measurement is the mean of 10 observations per leaf. LSD is the least significant difference to compare values within columns.

Hybrid	Stage (wk post-silking)	Year 1999	Year 2000	Year 2001
		SPAD		
Pride 5	2	38.8	41.4	46.6
	4	37.2	40.3	43.3
	6	34.2	37.6	42.0
Pioneer 3902	2	39.2	36.2	42.6
	4	37.1	39.9	43.9
	6	35.2	36.0	44.7
Pioneer 3893	2	40.7	38.7	44.1
	4	39.5	36.1	44.5
	6	38.1	36.7	45.2
	LSD (0.05)	5.6	4.4	3.7
		P-value		
Maize hybrid (H)		0.0871	0.0615	0.6658
Stage (S)		0.1351	0.1352	0.8271
H x S		0.1548	0.2366	0.0668

Table 3.2. Maximum quantum efficiency of PSII (Fv/Fm ratio) at three stages of development of three maize hybrids. LSD is the least significant difference to compare values within columns.

Hybrid	Stage (wk post-silking)	Year 1999	Year 2000	Year 2001
		Fv/Fm		
Pride 5	2	0.82	0.79	0.81
	4	0.81	0.76	0.76
	6	0.76	0.68	0.72
Pioneer 3902	2	0.81	0.78	0.81
	4	0.81	0.75	0.79
	6	0.79	0.73	0.74
Pioneer 3893	2	0.82	0.81	0.81
	4	0.83	0.77	0.78
	6	0.81	0.73	0.75
LSD (0.05)		0.02	0.01	0.02
		P-value		
Maize hybrid (H)		0.0037	0.0943	0.1447
Stage (S)		0.0081	0.0001	0.0001
H x S		0.3831	0.0025	0.7541

Table 3.3. Correlation coefficients between maximum quantum efficiency of PSII (Fv/Fm ratio) and its parameters (Fm and Fo) for three maize hybrids (Pride 5, Pioneer 3902 and Pioneer 3893).

Parameters	Hybrid Pride 5	Hybrid P-3902	Hybrid P-3893
	r		
Fm	0.31 *	0.27 *	0.26 *
Fo	0.05 *	0.13 *	0.10 *

* significant at 0.05

Leaf photosynthesis in fully illuminated plants gradually declined during the grain-filling period in all three growing seasons (Table 3.4) and the magnitude of the decline was greater in the older than in the newer hybrids. When averaged across growing seasons, decline in leaf CER from 2 to 6 wk after silking was 37% in Pride 5, vs. 20% in Pioneer 3902 and 18% in Pioneer 3893.

Table 3.4. Leaf carbon exchange rate (CER) at three stages of development of three maize hybrids. LSD is the least significant difference to compare values within columns.

Hybrid	Stage (wk post-silking)	Year 1999	Year 2000	Year 2001
		CER (μmol CO_2 m^{-2} s^{-1})		
Pride 5	2	37.0	29.7	47.4
	4	30.3	30.0	41.9
	6	23.2	18.6	34.1
Pioneer 3902	2	37.7	30.6	47.1
	4	36.4	29.3	42.9
	6	29.3	24.7	38.5
Pioneer 3893	2	40.1	33.0	46.0
	4	35.3	32.6	43.4
	6	29.9	25.2	42.3
	LSD (0.05)	7.3	5.1	4.3
		P-value		
Maize hybrid (H)		0.0285	0.0316	0.0571
Stage (S)		0.0001	0.0001	0.0001
H x S		0.7569	0.1370	0.0050

As with leaf CER, leaf conductance declined from 2 and 6 wk after silking (Table 3.5) and, when averaged across growing seasons, the magnitude of that decline was 42%

(Pride 5), 40% (Pioneer 3902) and 38% (Pioneer 3893). However, no difference in the decline among hybrids was observed. The absence of a concomitant decline of leaf CER and leaf COND indicates that factors other than stomatal effects could be involved in the differences in the decline in leaf CER among hybrids during the grain-filling period. When averaged across growing seasons and hybrids, leaf COND declined 38% between 2 and 6 wk after silking (Fig. 3.1A). When leaf COND was averaged across growing seasons and stages, values were significantly greater in the newer hybrids than in the old one (Fig. 3.1B).

Diurnal changes in leaf CER, COND and Fv/Fm ratio

When morning and afternoon values of leaf CER were compared, a reduction during afternoon was detected in all three hybrids throughout the grain-filling period and the magnitude of the afternoon decline did not change as the grain-filling period progressed. When averaged across hybrids and growing seasons, the afternoon reductions in leaf CER were 13% and 7% at 2 and 6 wk after silking, respectively (Table 3.6). The afternoon reduction in leaf CER was also observed in plants that were placed under a 30% shade during the day (Table 3.5). Diurnal changes in leaf CER were accompanied by concomitant changes in leaf COND in both control and shade treatments (Table 3.6).

In general the Fv/Fm ratio during the day was not different from that at sunrise. The only exception was in the 2001 growing season at 6 wk after silking, when Fv/Fm ratio declined during the day and decline started earlier in Pride 5 than P-3902 and P-3893 (Fig. 3.2A). The diurnal changes in Fv/Fm ratio were associated more with changes in Fm than in Fo (Fig. 3.2B) and, consequently, diurnal differences in the Fv/Fm ratio between older and newer hybrids seem be the result of a differential ability to dissipate heat rather than photoinhibition (Maxwell and Johnson, 2000).

Table 3.5. Leaf conductance at two stages of development of three maize hybrids. LSD is the least significant difference to compare values within columns.

Hybrid	Stage (wk post-silking)	Year 1999	2000	2001
		COND (mmol m^{-2} s^{-1})		
Pride 5	2	295	319	430
	6	169	200	234
Pioneer 3902	2	399	294	457
	6	259	167	261
Pioneer 3893	2	326	472	428
	6	230	250	321
	LSD (0.05)	73	72	53
			P-value	
Maize hybrid (H)		0.1023	0.0134	0.4126
Stage (S)		0.0024	0.0001	0.0001
H x S		0.8783	0.4187	0.2783

Table 3.6. Leaf carbon exchange rate (CER) and stomatal conductance measured at morning (10 am) and afternoon (2 pm) during two stages (2 and 6 wk after silking), in fully illuminated and shaded plants. Means across 3 years and three maize hybrids.

Treatment	Stage (wk post-silking)	CER		Conductance	
		Morning	Afternoon	Morning	Afternoon
		µmol CO_2 m^{-2} s^{-1}		mmol CO_2 m^{-2} s^{-1}	
Control	2	38.6 a*	33.7 b	380 a	279 b
	6	29.7 a	26.8 b	232 a	202 b
Shade	2	37.1 a	29.8 b	295 a	232 b
	6	28.4 a	27.3 a	211 a	178 b

* Values within the same row for each variable followed by the same letter are not significant different ($P > 0.05$).

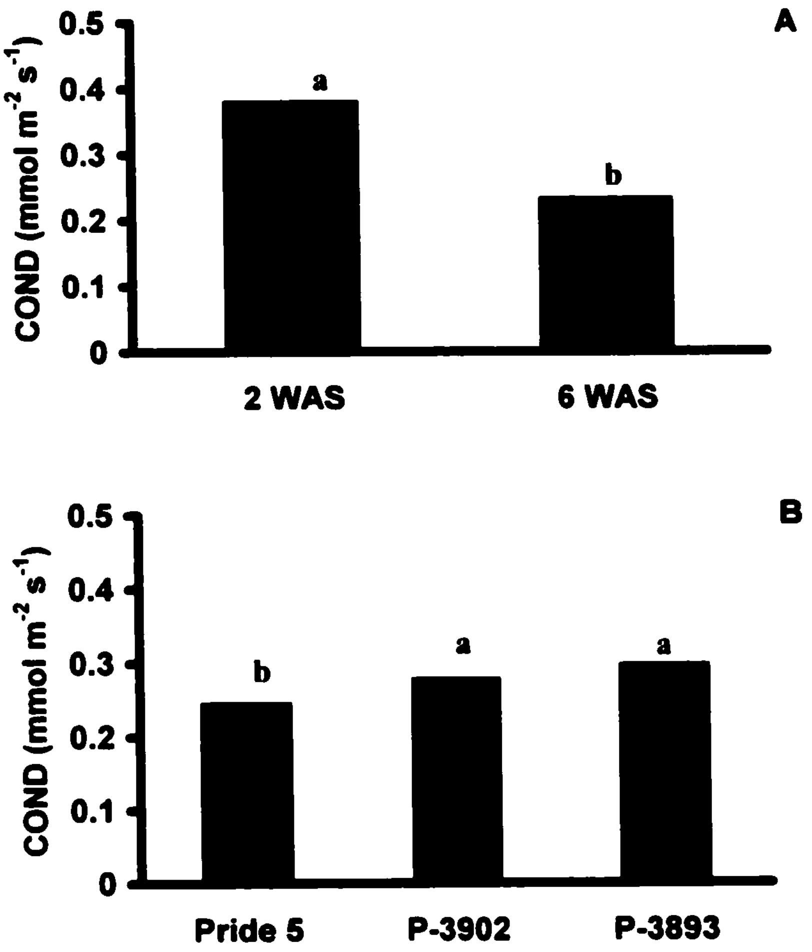

Fig. 3.1. Leaf conductance (COND) at 2 and 6 weeks after silking (WAS) (A) and for three hybrids (B). Data are means across years and hybrids in A and across years and stages of development in B. Bars with different letters are significantly different. based upon LSD (0.05).

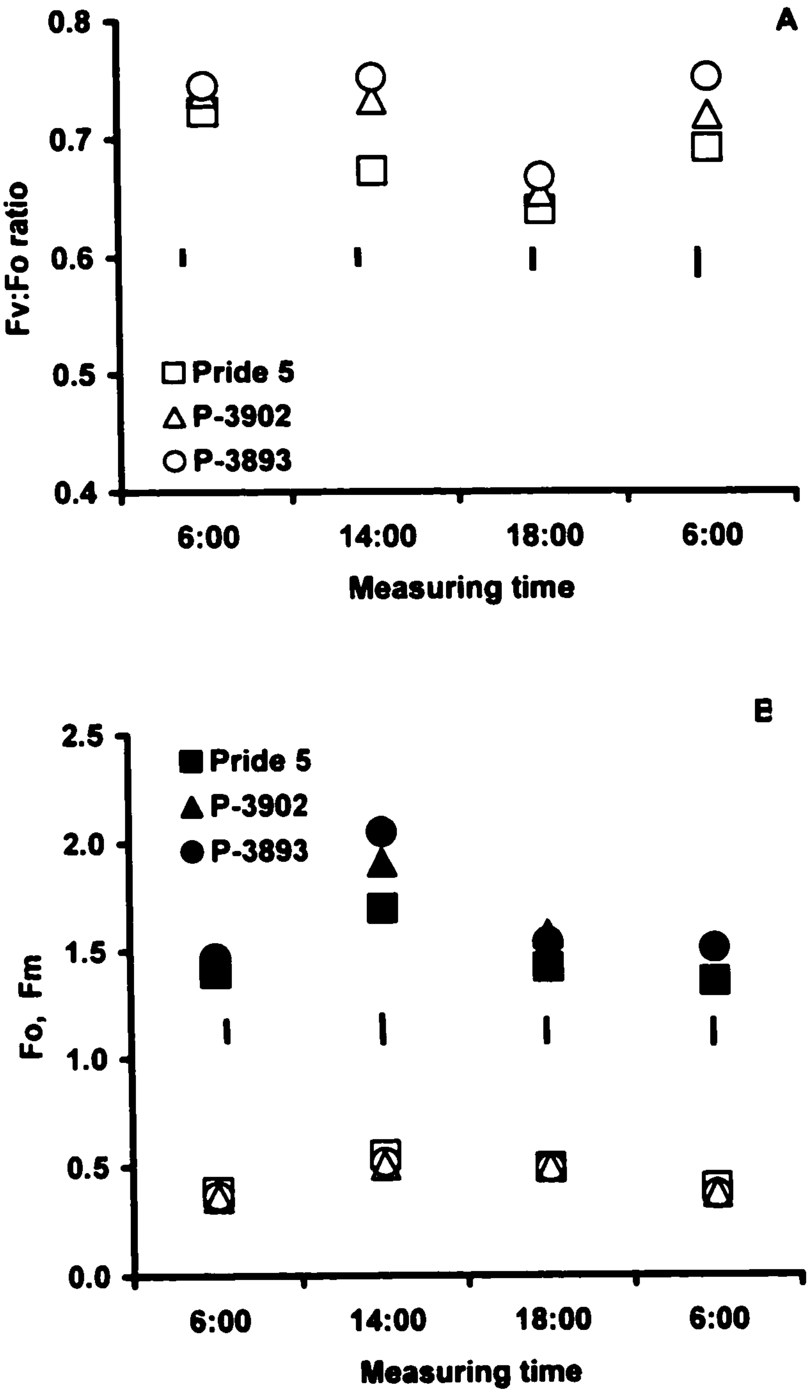

Fig. 3.2. Diurnal changes in maximum quantum efficiency of PSII (Fv/Fm ratio, A) and its components Fm and Fo (B) for three maize hybrids (Pride 5, Pioneer 3902, and Pioneer 3893) at 6 wk after silking in year 2001. Measurement were made at 6:00, 14:00, 18:00, and 6:00 hr next day. In B, open and filled symbols represent Fo and Fm, respectively.

Bars represent LSD (0.05) for comparing hybrids within measuring time.

DISCUSSION

The main objective of the present work was to examine leaf senescence in terms of its functional symptoms in an older and two more recent maize hybrids during the grain-filling period. Results support the contention that the improved performance during the grain-filling period in newer vs. older hybrids is associated with a more active green leaf area index. The more active green leaf area in new hybrids was the result of a delay in the decline in functional symptoms of leaf senescence such as leaf CER and Fv/Fm ratio.

Functional symptoms of leaf senescence (i.e., leaf CER, leaf COND, and Fv/Fm ratio) might account for differences in grain yield between older and newer hybrids when these are not fully explained by differences in light absorptance (Tollenaar and Aguilera, 1992). Indeed, functional symptoms of senescence occurred in leaves in which chlorophyll content had not changed. Results of this study enhance the concept of leaf senescence by adding functional symptoms of leaf senescence to visual symptoms of leaf senescence.

The relationships between *stay green* and photosynthesis, and *stay green* and grain yield, have been analyzed in other grass species. Mutants of fescue that were able to maintain green leaves until maturity have been reported to show a decline in leaf CER with age (Thomas, 1987) and genotypes of sorghum that remain green until advanced stages in the grain-filling period have shown significant differences in yield (Borrell et al., 2000), 1996). In agreement with those reports, results of the present study indicate that maize hybrids, despite staying green up to advanced stages of life cycle, undergo a decline in their photosynthetic capacity that, eventually, would result in lower grain yield. Thomas and Howarth (2000) used the expression "cosmetic" *stay green* to define genotypes that, although staying green until maturity, show a decline in their photosynthetic capacity. To the best of our knowledge, a score indicating a level of agreement between leaf CER and leaf greenness in current commercial hybrids has not been developed and could be useful in identifying the physiological basis for genotypic differences in grain yield.

In this study, newer hybrids retained higher levels of leaf CER after silking and these results concur with those reported for other field crops such us soybean and rice (Wells et al., 1982; Sasaki and Ishii, 1992) where a slower decline in photosynthetic activity was reported for modern cultivars when compared with old cultivars. Differences in leaf CER between the older and newer hybrids become greater as the growing season progresses after flowering. For instance, Nissanka (1995), did not find differences in canopy photosynthesis by tasseling time and Ying et al. (2000) found similar photosynthetic rates in both older and newer hybrids at silking. Differences in the magnitude of leaf CER decline between the older and newer maize hybrids tended to appear as early as 4 wk after silking and the differences in CER became significant at 6 wk after silking. Moreover, differences between both groups of hybrids could be greater if plants were grown at higher plant densities (Dwyer et al., 1991) than that used in our hydroponic experiment. Maintenance of high leaf CER late in the growing season is apparently one of the traits associated with greater dry matter accumulation, and, consequently, with greater yield observed in more recent hybrids when compared with older hybrids (Earl, 1998). Tollenaar and Aguilera (1992) reported greater crop growth rates between silking and 6 wk after silking in newer hybrids compared to an older hybrid. Greater crop growth rates, and consequently, greater dry matter accumulation during the grain-filling period in more recent hybrids have been attributed in a large part to an enhanced efficiency to convert intercepted radiation in dry matter (Tollenaar and Aguilera, 1992). Based on our results, we postulate that the capability to sustain leaf CER during the grain-filling period plays an important part in the enhanced efficiency to convert light to dry matter, and eventually, to the greater grain yield of more recent hybrids. Even though a consistent relationship between grain yield and photosynthesis is not always evident (Crosbie et al., 1977; Charles-Edwards, 1978), our postulate is consistent with other reports where hybrids with high grain yield maintained high photosynthesis after silking (Crafts-Brandner et al., 1984a; Hageman and Below, 1984).

Interestingly, the progress of functional symptoms of senescence occurred without a significant decline in chlorophyll content as estimated by the SPAD reading. Leaves attached to the central section of the plant, where measurements were made, stayed healthy and green throughout the grain-filling period, which constitutes an indication of high light absorptance (Earl and Tollenaar, 1997). When a rather stable but high absorptance level is accompanied by a declining leaf CER as the growing season progress, an alternative(Table 3.5) mechanism for dissipating the excess of absorbed energy in a harmless way is necessary. It has been suggested that the light demanding process by which zeaxanthin pigment is formed in the xanthophyll cycle plays a mayor role in the dissipation of that excess (Demming-Adams and Adams, 1992), and that changes in the final destination of reducing equivalents are reflected by a decline in the maximum quantum efficiency at PSII. This in turn is related to a reduction in the Fv/Fm ratio. In agreement with that, our results are indicative of a decline in the maximum quantum efficiency of PSII.

The reduction in the Fv/Fm ratio constitutes another symptom of functional leaf senescence. Large reductions in this ratio associated with leaf senescence have been reported by Lu et al. (2001) in wheat (*Triticum aestivum* L.), and seasonal reductions in the Fv/Fm ratio have been reported by Lu and Zhang (1998) in maize plants grown outdoor in pails with adequate water and nutrients. Fluorescence techniques have been successfully used in assessing photosynthetic performance and capturing differences among maize genotypes under abiotic and biotic stress conditions (Tollenaar and Mihajlovic, 1991; Ying et al., 2000) and under field conditions (Earl and Tollenaar, 1999). In agreement with those reports, the differential decline of the Fv/Fm ratio observed during the grain-filling period between the older and newer hybrids suggests that this ratio represents a tool to discriminate among maize hybrids in the absence of stressful conditions. Previous work has indicated that chlorophyll fluorescence measurements might be used as a selection tool to improve photosynthetic performance in breeding programs (Araus et al., 1998; Fracheboud et al., 1999). Ying et al. (2000) suggested that one cause of the CER reduction is a decline in the maximum quantum efficiency of PSII. In our study, in spite of concomitant changes in both leaf CER and the Fv/Fm ratio during the last part of growing season, the association between these two measurements was not significant; however Fm was significantly associated with leaf CER (not shown). In fluorescence analysis, Fm is used to refer the maximum chlorophyll fluorescence in dark-adapted state and is generally assumed as reflecting the ability for heat dissipation (Maxwell and Johnson, 2000). Hence, the delayed declined of leaf CER observed in newer hybrids might be in part associated with an enhanced ability to dissipate heat from the PSII reaction centre.

Beside the seasonal changes in leaf CER, a depression in leaf CER during the afternoon relative to that during the morning was observed. This depression occurred despite a comparable level of irradiance, and was also detected when maize plants were placed in 30% shade. Afternoon depression of leaf CER in maize has been previously reported

(Bunce, 1990; Hirasawa and Hsiao, 1999), but to the best of our knowledge, comparisons among maize hybrids have not been made. The magnitude of the afternoon decline of leaf CER was similar for all three hybrids. Afternoon depression has been predominantly associated with closing stomata and water vapour deficits. For instance, Hirasawa and Hsiao et al. (1999) attributed afternoon depression of leaf CER to stomatal effects and Bunce (1990) attributed it to a transient leaf water deficit resulting from high transpiration induced by high water vapor-pressure deficit, which occurred even with an ample supply of water. In the study reported herein we found that afternoon depression of leaf CER was paralleled by an afternoon decline of leaf COND, which capture stomatal effects.

In general, we did not find a reduction in the Fv/Fm ratio in most days when measurements were performed. However, the number of measurements per day may have been insufficient to capture changes in the Fv/Fm ratio. Indeed, when the number of daily measurements per day was increased in the 2001 growing season, an afternoon reduction in the Fv/Fm ratio was observed and, at 6 wk post-silking, the reduction in the Fv/Fm ratio was greater in Pride 5 than P-3902 and P-3893. Similar to the trend across the growing season, the changes in the Fv/Fm ratio were associated more with changes in Fm than with Fo (Figure 3.2B). Thus, one could speculate that diurnal differences in the maximum quantum efficiency of PSII between older and more recent maize hybrids are associated more to a differential enhancing in xanthophyll cycle-related thermal dissipation than to differential susceptibility to photoinhibition related to photodamage in the in the PSII antennae (Demming-Adams and Adams, 1992). Nevertheless more research is needed to substantiate this conjecture.

CONCLUSIONS

Delaying leaf senescence has been a prime target for crop improvement (Thomas and Howarth, 2000), and it continues to be an avenue for increasing the total amount of carbon fixed by maize crops. Results of this study demonstrated that reported differences in dry matter accumulation, and consequently grain yield, between older and more recent maize hybrids could be accounted by differential progress in functional symptoms of leaf senescence during the grain-filling period. Functional symptoms of leaf senescence were expressed as a decline in leaf CER as growing season progressed, and the rate of decline was steeper in the older than in newer hybrids. A similar trend was apparent for the Fv/Fm ratio. Although the Fv/Fm ratio was poorly associated with CER, one of its components (Fm) had a significant correlation with CER, indicating that a slower seasonal decline in leaf CER in newer hybrids might be associated, at least in part, with an increased ability to promote thermal dissipation of the energy from excited chlorophyll molecules.

During the day, the decline in leaf CER from the morning to afternoon was similar for all hybrids and the decline was associated with a decline in leaf COND. However, this afternoon decline of leaf CER and COND did not increase as senescence progressed. In addition, reductions in the Fv/Fm ratio were rarely detected during the afternoon and, when detected, the reductions were transient and almost completely reversed by the next morning. Although the agronomic impact of a diurnal decline in photosynthetic performance is hard to predict, our results were successful in identifying instances when the leaf CER is less than expected, which probably represents a resource to be exploited in the future for increasing photosynthesis at the canopy level.

Although future yield improvement in maize may involve a reduction in the rate of leaf senescence during the grain-filling period, the greatest potential for yield improvement is in maintaining leaf photosynthetic rate during the grain-filling period. Genetic differences in leaf photosynthesis seem to be related more to seasonal than daily fluctuations.

CHAPTER IV

EFFECT OF GENOTYPE, NITROGEN, PLANT DENSITY, AND ROW SPACING ON THE AREA PER LEAF PROFILE IN MAIZE

ABSTRACT

The distribution of area per leaf along the stem of a maize plant can be described by a bell-shaped function ($LA_n = y_0 * exp[-b*(x_n-x_o)^2 + c*(x_n-x_o)^3]$) that can be used to estimate the area of a leaf at any position (LA_n) from the area (y_o) and position of the largest leaf (x_o) from bottom to top. The objectives of this study were i) to examine the effect of nitrogen, hybrid, plant density and row spacing on the area-per-leaf profile by analyzing the coefficients that define this bell-shaped function, ii) to describe the routine used to compute total leaf area and the area per leaf profile in the MAIS crop simulation model and iii) to compare the area-per-leaf profile calculated by the model with those measured in the field in an independent set of data. Four data sets from experiments that included different plant densities, nitrogen levels, hybrids, and row spacing were used. In all experiments the area of each individual leaf was measured on five plants per plot, averaged, and normalized with the corresponding value of the largest leaf. For each plot, coefficients of the bell-shaped function, that quantify the 'breadth' of the area-per-leaf profile (b), the degree of skewness (c), and the position of the largest leaf (x_o) in the bell shape function were estimated for by using non-linear regression. Once normalized, observations fitted well to the bell-shaped function (i.e., $r^2 > 0.95$) and coefficients were compared by using analysis of variance. Two out of three coefficients showed small but significant variations for the factors examined. Coefficient b decreased under high soil-N level, high plant density, and was lower for a newer than an older hybrid, whereas the opposite occurred with coefficient x_o. Row spacing only affected x_o in one data set. Mean temperature and accumulated PAR during a period of 30 days after planting were

associated with coefficient b and x_o in a linear and quadratic fashion, respectively. Coefficient c was not significantly altered by any factor. Once a general equation of the bell-shaped function with average values for the coefficients was incorporated in the model MAIS, there was good agreement between the area-per-leaf profile estimated by the model and that independently observed in the field. Our results confirm the robustness of the normalized bell-shape function in describing the area-per-leaf profile and indicate that variations inherent to year, nitrogen, plant density, hybrid and row spacing on full leaf area can be quantified adequately by measuring their effects on the area and position of the largest leaf.

INTRODUCTION

Accurate estimation of leaf area is an essential part in models that simulate crop growth and yield because leaf area influences the percentage of solar radiation intercepted and, consequently, dry matter accumulation and grain yield. In crop growth simulation models that compute dry matter accumulation from temporal and spatial integration of photosynthesis, rate of leaf expansion, maximum area and rate of leaf senescence of each individual leaf are important factors in the estimation of canopy photosynthesis. In addition, when sunlit/shade leaf area and LAI by layers are considered in calculating canopy photosynthesis (Boote et al., 1996), estimations of the area-per-leaf profile may be required.

In the model MAIS (Drost, 2001), a crop growth simulator for maize (*Zea mays* L.) grown under non-limiting soil conditions, the growth of each individual leaf is determined by the time of its appearance, and the duration and rate of its expansion (Stewart and Dwyer, 1994a), LAI is computed as the sum of the areas of all leaves. Duration and rate of expansion are described by non-linear functions of leaf number (Stewart and Dwyer, 1994a) and, consequently, the area of any leaf (LA_n) may also be plotted as a function of leaf number. The distribution of leaf area by position conforms to a slightly skewed bell-shaped curve (Dwyer and Stewart, 1986b). This function for any leaf number n is described by the equation $LA_n = y_0 * exp[-b*(x_n-x_o)^2 + c*(x_n-x_o)^3]$, where the amplitude ($y_0$) represents the size of the largest leaf, the point of inflection (x_o) is the leaf number (x_n) of the largest leaf, and b and c control the degree of breadth and skewness of the area-per-leaf profile, respectively. The four coefficients defining the bell-shape function can be biologically interpreted (Keating and Wafula, 1992) and, consequently, are useful in analyzing changes in the area per leaf profile inherent to growing seasons and agronomic practices. Moreover, by knowing the magnitude of changes in those coefficients, eventual changes in the pattern of light interception can be quantified.

Coefficients defining the bell-shaped function have been studied in both temperate and tropical maize. In temperate maize, Dwyer and Stewart (1986b) compared the area-per-leaf profile of six data sets representing three growing seasons by normalizing the bell-shaped function. They found little year to year variation in b, c, and x_o coefficients, and their results showed that the prediction of the total LAI and area-per-leaf profile could be estimated from single estimations of the largest leaf. In a tropical maize open pollinated population, Keating and Wafula (1992) studied the area-per-leaf profile under a range of total number of leaves per plant (TNLEAF). They found that coefficients of the bell-shaped function could be estimated from the total number of leaves per plant. Results of both studies indicate that the area-per-leaf profile could be simulated from a relationship between TNLEAF and y_o. The wide range of TNLEAF that was reported in the study of tropical maize genotypes (i.e., 12 to 17) is not common in temperate maize hybrids. A single relationship using TNLEAF as the independent variable does not seem adequate to estimate coefficients of this function in single cross hybrids.

Crop models have been used in research to analyze crop growth response to different agronomic practices (Boote et al., 1996). Even though the effects of changes in agronomic practices (such as hybrid, nitrogen, plant density and row spacing) on simulated yield and LAI are frequently reported in the literature, to the best of our knowledge their impact on the area-per-leaf profile has not been reported. Coefficients of the bell-shaped function could capture those changes that, once quantified, would be useful to test the robustness of the this function in estimating the area-per-leaf profile in maize.

The primary objective of this study was to examine the impact of nitrogen, hybrid, plant density and row spacing on the area-per-leaf profile by analyzing the coefficients that define the bell-shaped function. Secondly, the effect of changes in these coefficients on simulated LAI and the leaf area profile was estimated by the model MAIS and compared with measured values in the field.

MATERIALS AND METHODS

Experimental procedures

Four field studies were carried out at the Woodstock and Elora Research Stations (Ontario, Canada) between 1997 and 2001 (Table 4.1).

Table 4.1. Data sets where the area of each individual leaf was measured.

Data Set	Location	Year	Nitrogen (kg ha^{-1})	Plant density (plants m^{-2})	Row spacing (cm)	Hybrid
1	Woodstock	1997 - 2000	ne†	ne	51-76-76 T‡	Pioneer 3893
2	Woodstock	1999 - 2000	ne	6.9 - 8.9	ne	Pioneer 3893
3	Woodstock	1999 - 2000	0 - 180	ne	51-76-76 T	Pioneer 3893 Pioneer 39P06
4	Elora	2001	Ne	3.5 - 12	ne	Pride 5 Pioneer 3902 Pioneer 3893

† ne: data not evaluated
‡ Twin rows

In Woodstock, all experiments were machine planted at 7 plant m^{-2}, and weed and pests were chemically controlled. When N was not a factor to be tested, plots were fertilized with N according to soil test and recommendations. When the factor plant density was tested, the final number of plants per unit area was established by thinning. In Elora, all plots were hand planted and thinned to the target plant density and weeds and pests were chemically controlled. In short, data sets included i) the hybrid Pioneer

3893 grown at three row spacings, 51 cm (RS1), 76 cm (RS2) and 76 cm in twin-rows (RS3) during four years (1997 to 2000), ii) Pioneer 3893 grown at two plant densities (6.9 and 8.9 plant m^{-2}) during two years (1999 and 2000), iii) the hybrids Pioneer 3893 and Pioneer 39P06 grown at two levels of nitrogen amendments (0 and 180 kg ha^{-1}) and three row spacings (RS1, RS2, and RS3) during two years (1999 and 2000), and iv) the hybrids Pride 5, Pioneer 3902, and Pioneer 3893 grown at two plant densities (3.5 and 12 plant m^{-2}) during one year (2001). The experimental design was a randomized complete block with four replications for Data Set 1 (DS1) ((i) above) and a randomized complete block in a split plot arrangement with four replications for the other data set (DS2, DS3 and DS4) (ii, iii and iv above). Length and maximum width of all leaves on five consecutive plants in a row per plot were measured and the leaf area of each individual leaf calculated by multiplying length by maximum leaf width by 0.75 (Montgomery, 1911). Measurements of length and width were performed three occasions during the period from plant emergence to silking in order to perform measurements on all newly expanded leaves before senescence or breakage occurred.

The area of each individual leaf was normalized with respect to the largest leaf. For each plot, coefficients b, c, and x_o of the bell-shape function were estimated using non-linear regression performed in the Proc N-LIN of SAS (SAS, 1997). Once observations fitted well to the bell-shaped function (i.e., $r^2 > 0.95$) for each data set, coefficients were compared by using an analysis of variance performed in the PROC-GLM of SAS.

In order to compare values of measured leaf area and values predicted from the bell-shaped function (i.e., from the area of the largest leaf), three independent sets of data were used. Data sets included area-per-leaf (mean of five plants) of 1) Pioneer 3893 grown at the three row spacings, 2) Pioneer 3893 grown at 5.1 and 7 plants m^{-2}, and 3) hybrids Pride 5, Pioneer 3902 and Pioneer 3893 grown at 1 plant m^{-2}.

After the bell-shaped function was incorporated in the subroutine LEAFAREA of the model MAIS, using the mean values of each of the coefficients in the four data sets, values of area-per-leaf profiles were estimated and compared to those observed in an independent set of data.

Model description

A detailed description of the model MAIS can be found in Drost (2001). In short, the model consists of three main components: i) phenology, ii) dry matter accumulation and iii) dry matter partitioning.

i) Phenology: During the vegetative period, the length of the planting-silking period is determined by the total number of initiated leaves, the rate of leaf appearance, and the duration between the emergence of the topmost leaf and silking. The effect of temperature on rate of development between planting and silking is quantified by the rate of leaf tip appearance (RLA). RLA is estimated as the mean of rates at the daily minimum and maximum temperatures, using an equation derived from results of growth cabinet studies (Tollenaar et al., 1979): $RLA = 0.0997 - 0.03360 * T + 0.0036 * T^2 - 0.0000639 * T^3$, where T is either the daily maximum or minimum temperature. The rate of leaf appearance between the 3- and 12-leaf stage is modified by incident solar radiation (Tollenaar, 1999). Total number of initiated leaves (TNLEAF) is used by the model to quantify genotypic and abiotic factors influencing duration of the life cycle. For a specific genotype, the basic leaf number (i.e., number of initiated leaves under a photoperiod of 12 hours and an air temperature of 15 °C) is increased when the photoperiod is greater than 12 hours and air temperature is higher than 15 °C during the period when leaf number is sensitive to photoperiod and temperature (Tollenaar and Hunter, 1983). This period extends from the 4-leaf stage to tassel initiation, which occurs in early temperate germplasm at 0.46 times the total number of initiated leaves (Gay, 1980; Tollenaar and Hunter, 1983). TNLEAF increases as photoperiod increases between 12 and 16 hours, and the rate of increase is genotype specific. For instance, when photoperiod varied from 12 to 16 hours, the number of leaves added to the basic leaf number in short-season hybrids grown in Ontario (Tollenaar and Hunter, 1983) is 2.6. TNLEAF increases also by 0.2 leaves per °C above 15 °C (Tollenaar and Hunter, 1983) and by 0.4 leaves per °C below 15 °C (Warrington and Kanemasu, 1983).

ii) Dry matter accumulation: dry matter accumulation is calculated in a canopy photosynthesis subroutine that requires LAI as an input to estimate the absorption of incident photosynthetic photon flux density (PPFD). Leaf photosynthesis (P_L) is computed by using negative exponential curve, $P_L = P_{max} \times [1 - \exp(-I_A \times \alpha/P_{max})]$, where P_{max} is the PPFD saturated rate of leaf photosynthesis, I_A is absorbed PPFD per unit leaf area, and α is the photosyntetic efficiency at low PPFD (EFF). EFF is set at 0.45 kg CO_2 ha^{-1} $hour^{-1}$ per J m^{-2} s^{-1}, which is equal to 0.0606 mol CO_2 (mol photon)$^{-1}$. Canopy photosynthesis for a day is estimated by computing photosynthesis across the day. In addition, leaf photosynthesis of sunlit and shade leaf area are computed separately by each layer according to the proportion of leaf area that is sunlit and shaded. Once canopy photosynthesis is computed (i.e., gross photosynthesis), maintenance and growth respiration are subtracted to calculate net canopy photosynthesis and rate of dry matter accumulation.

iii) Dry matter partitioning: the fraction of dry matter partitioned to various plant components are calculated in a subroutine that allocated dry matter to different parts as a function of stage of development. Partitioning coefficients were derived empirically from growth chamber and field experiments.

Leaf area index in the model MAIS is estimated by calculating the expansion of the area of each individual leaf from the bottom of the plant to the top. The bell-shaped function is used to relate the area of each individual leaf to its position on the stem (Dwyer and Stewart, 1986b). Rate of leaf area expansion is influenced by stage of development and temperature, and the model uses empirical relationships determined from the results of a growth-cabinet study between LAI and both stage of development and temperature (Tollenaar, 1989b). Each day, the leaf area expansion of all expanding leaves is computed by a five-step numerical integration, beginning at tip emergence and ending at leaf-collar appearance (Stewart and Dwyer, 1994a; 1994b). The assumption in the model is that leaf area per plant prior to the 12-leaf stage is not influenced by assimilate supply *per se* (Tollenaar, 1999). The impact of plant density on leaf area is based on effect of mutual shading on leaf area per plant at the 12-leaf stage (Tollenaar, 1992; Tollenaar et al., 1994b). Once leaf expansion is completed, leaf senescence is computed according to leaf age and leaf position (Tollenaar and Daynard, 1978). The rate of leaf senescence is accelerated by stress, which is quantified as a decline in the rate of dry matter accumulation per unit leaf area.

RESULTS

Estimation of coefficients of bell-shape function

There was an excellent agreement between normalized observations and the function describing the bell-shape function ($r^2 > 0.95$) for the variables year, plant density, row spacing, nitrogen and hybrid. As expected once data were normalized, the coefficient indicating the area of the largest leaf (y_o) was close to 1.0. Analyses of variance for b, c and x_o coefficients are shown in Table 4.2. Coefficient c, which describes the degree of skewness in the area-per-leaf profile, was not affected by any factor included in our data sets. Therefore, our analysis was focused on the other two coefficients of the bell-shape function (b and x_o) that were significantly altered by at least one environmental factor.

Alterations in coefficient b and x_o of the bell-shape function

Year, plant density and hybrid had a relatively large impact on the coefficients, whereas the effect of nitrogen and row spacing on the b coefficients was small. Analysis of variance showed significant effects of year on the b coefficient (Table 4.2). The variation between maximum and minimum values of b due to years was 18% in Data Set 1, 6% in Data Set 2, and 13% in Data Set 3 (Table 4.3). Analysis of variance showed that plant density affected values of b only in the data set with a wide range of plant density. The value of b increased 16% as plant density increased from 3.5 to 12 plant m^{-2} in Data Set 4 (Table 4.3), whereas differences were non-significant in Data Set 2 when plant density was closer to the commercial range (6.9 and 8.9 plant m^{-2}).

Table 4.2. Analysis of variance of data sets studied. *b*, *c* and x_o are the coefficients of the bell shaped function normalized in respect to the largest leaf.

			Coefficients	
Data set	Source of variation	*b*	*c*	x_o
		----------P-value----------		
1	Year (Y)	0.0001	0.9135	0.0002
	Row spacing (RS)	0.9470	0.2125	0.6308
	Y x RS	0.8480	0.5397	0.1513
2	Year (Y)	0.0059	0.7843	0.0001
	Plant density (D)	0.1790	0.8399	0.0960
	Row spacing (RS)	0.9054	0.2631	0.3000
	Y x D	0.0299	0.3745	0.2488
	Y x RS	0.4824	0.0875	0.0653
	D x RS	0.1386	0.2112	0.5112
	Y x D x RS	0.8060	0.4441	0.1396
3	Year (Y)	0.0001	0.0872	0.9953
	Nitrogen (N)	0.0006	0.0832	0.0001
	Hybrid (H)	0.0001	0.8477	0.0001
	Row spacing (RS)	0.0676	0.4886	0.0099
	Y x N	0.1550	0.1587	0.9395
	Y x H	0.0145	0.2017	0.8198
	Y x RS	0.6949	0.2826	0.0409
	N x H	0.0345	0.3200	0.3665
	N x RS	0.8043	0.5652	0.1965
	H x RS	0.7856	0.2877	0.4240
	Y x N x H	0.4364	0.3093	0.0549
	Y x N x RS	0.8308	0.6590	0.5153
	N x H x RS	0.8016	0.4958	0.0005
	Y x N x H x RS	0.5357	0.7643	0.2946
4	Plant density (D)	0.0005	0.3108	0.0001
	Hybrid (H)	0.0014	0.3789	0.0001
	D x H	0.5569	0.0613	0.1039

Analysis of variance showed that hybrids differed significantly in respect to *b* values. In Data Set 3, the value of *b* was 13% greater for Pioneer 3893 than for Pioneer 39P06 and in Data Set 4, *b* was 19% greater for Pride 5 than for Pioneer 3893 (Table

4.3). Analysis of variance showed that row spacing did not significantly affect the value of *b* in two out three data sets (Data Set 1 and Data Set 2). However, in Data Set 3 the coefficient *b* was greater in plants established in twin rows ($p < 0.07$) than in plants established in single rows at a row width of 76 cm (Table 4.3). Analysis of variance showed that N significantly altered the values of *b* in the one data set in which soil N was varied. However, although N levels (0 vs. 180 kg ha^{-1}) resulted in significant differences in leaf area index and grain yield (data not shown), the difference in the *b* coefficient was 7% (Table 4.3).

The coefficient x_o was affected by year in two of the three data sets. These two data sets included 1998, a year characterized by high temperature at the beginning of growing season that resulted in larger number of leaves and, consequently, in a higher position of the largest leaf in 1998 than in the other years. Plant density significantly altered the values of x_o when a wide range of plant density was tested (Table 4.3), but no difference was found when 6.9 and 8.9 plants m^{-2} were compared. Hybrids varied significantly in relation to the position of the largest leaf probably as a result of differences in total number of leaves. The value of x_o was 12.5 in Pioneer 39P06 and 11.7 in Pioneer 3893 in Data Set 3. In Data Set 4, the largest leaf was positioned lower in Pride 5 than in Pioneer 3902 and Pioneer 3893 (11.4 vs.12.2). Similarly to that observed for coefficient *b*, row spacing altered the value of x_o in only one data set, i.e. Data Set 3, where small and significant differences were observed between RS2 and RS3 (12.0 vs. 12.2). The largest leaf was positioned in a higher position when nitrogen application increased from 0 to 180 kg ha^{-1} (11.9 vs. 12.3).

Table 4.3. Values of b and x_o coefficients in four sets of data. Only those factors with significant differences are shown. For each variable and column, different letters indicated significant differences within levels.

Data Set	Variable	Level	b	x_o
1	Year	1997	0.0369 d	11.59 b
		1998	0.0412 b	12.39 a
		1999	0.0382 bcd	11.79 b
		2001	0.0451 a	11.91 b
2	Year	1997	0.0375 b	11.50 b
		1998	0.0398 a	12.50 b
3	Year	1999	0.0368 b	12.09 a
		2000	0.0423 a	12.09 a
	Nitrogen	0 kg ha^{-1}	0.0409 a	11.93 b
		180 kg ha^{-1}	0.0381 b	12.26 a
	Hybrid	Pioneer 3893	0.0422 a	11.72 b
		Pioneer 39P06	0.0368 b	12.47 a
	Row Spacing	51 cm	0.0399 ab	12.09 ab
		76 cm	0.0404 a	11.96 b
		76 cm (twin)	0.0383 b	12.23 a
4	Plant density	3.5 plant m^{-2}	0.0383 b	12.20 a
		12 plant m^{-2}	0.0456 a	11.64 b
	Hybrid	Pride 5	0.0469 a	11.36 b
		Pioneer 3893	0.0378 b	12.28 a

In spite of significant changes observed in b and x_o among different treatments, the impact *per se* of b and x_o on leaf area per plant (expressed as the sum of all normalized values of leaf area) was small. For instance, even though the area-per-leaf profile in Pioneer 3893 was flatter than in Pride 5, as reflected by 19% reduction of b value, the increase in leaf area per plant attributable to a flatter area profile was not greater than 10% (Table 2.4). Similarly, maximum changes in the position of the largest leaf due to hybrids (8%) had little effect on total the leaf area per plant (Table 4.4).

Table 4.4. Maximum changes in bell shape function coefficients (b, x_o) and their respective impact on the total leaf area per plant. Changes in leaf area were computed by assuming a plant with 19 leaves. When changes in b were evaluated, x_o was the mean of all four sets of data and vice versa.

	Changes			
	b	Total leaf area †	x_o	Total leaf area ‡
	----------------------------Change from mean (%)			--------------------------
Year	18	9.5	6.4	0.9
Nitrogen	7	3.4	2.7	0.3
Hybrid	19	10	7.5	0.9
Plant density	16	8.3	4.6	0.6
Row spacing	5	2.4	2.2	0.6

† Calculated as the sum of normalized area of all leaves.
‡ Normalized with respect to the largest leaf.

Validation of a general bell shape function

Because of the small impact of variations in b and x_o on total leaf area in addition to low sensitivity of the c coefficient to the environmental variables, it seems reasonable that a general bell-shape equation (henceforth called the general equation) with mean values of b, c, and x_o (i.e., averaged across our four data sets) could be utilized in estimating the area per leaf profile when the area of the largest leaf (i.e., the absolute value of y_o) is known. We used this approach to compare the area of 544 individual leaves measured in the field with their respective area estimated by the general equation and the area of the largest leaf. Although the linear relationship was significant ($P < 0.05$, $r^2 = 0.93$), the slope was lower than 1 (Fig. 4.1), indicating that the general equation underestimated the observed values. Estimated values were still slightly lower than the observed values when the independent set of data was divided into groups differing in row spacing, plant density and hybrid (Table 4.5).

Simulation of area-per-leaf profile from model MAIS

Once that general equation was incorporated into the subroutine LEAFAREA of model MAIS, a comparison between observed and simulated values of individual leaf area was performed. Observed values came from an available data set which included measurement of leaf area from Leaf 7 to Leaf 18 of the maize hybrid P-3893 grown at 3.5 and 12 plant m^{-2} at the Elora Research Station during 1999, a year without apparent below-ground limitations. There was a good agreement between observed and simulated values at both 3.5 and 12 plant m^{-2} (Fig. 4.2). However, the simulated leaf area was lower than the observed leaf area at a plant density of 12 plant m^{-2} as indicated by a slope < 1 (Fig. 4.2b).

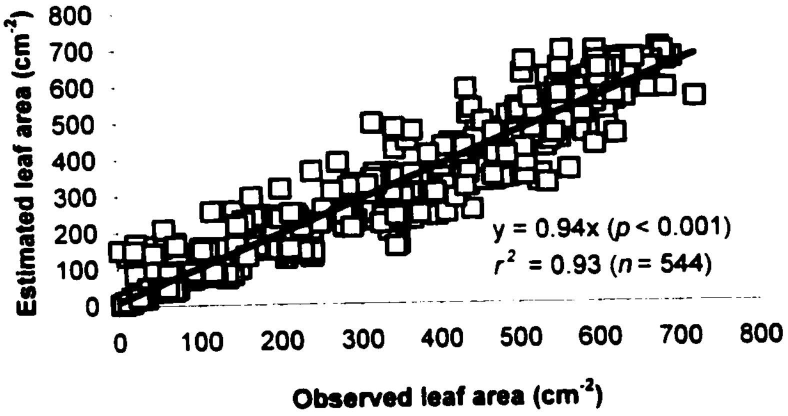

Fig. 4.1. Observed values of individual leaf area compared with those estimated from the bell-shape function. For each individual leaf, its area (LA_n) was estimated as $LA_n = y_o * \exp(0.04019*(x_n-12)^2 + 0.00015*(x_n-12)^3$, where y_o was the area of the largest leaf and x_n was the leaf number of the leaf n.

Table 4.5. Slope and coefficient of determination of each linear relationship between observed and estimated values in three independent data sets.

Data set	Location	Year	Factor	Slope	r^2
1	Woodstock	1997	Row spacing	0.95	0.94
			51 cm	0.95	0.95
			76 cm	0.97	0.90
			76 cm (T) †		
2	Woodstock	1998	Plant density		
			6.9 plant m^{-2}	0.95	0.94
			8.9 plant m^{-2}	0.97	0.94
3	Elora	2001	Hybrid		
			Pride 5	0.94	0.95
			Pioneer 3902	0.93	0.94
			Pioneer 3893	0.91	0.91

† Twin rows

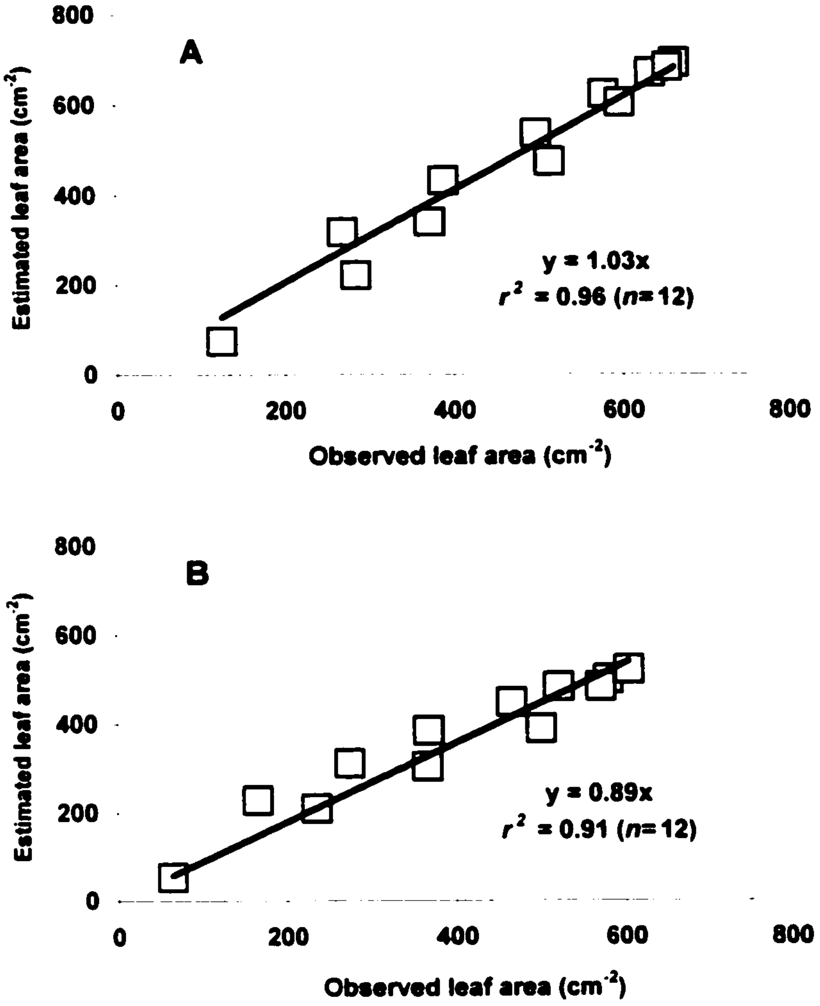

Fig. 4.2. Leaf area observed vs. leaf area estimated by model MAIS for individual leaves in Pioneer 3893 during 1999 at (A) 3.5 plant m^{-2} and (B) 12 plant m^{-2}.

DISCUSSION

The primary objective of this study was to evaluate changes in the area-per-leaf profile in response to a range of year conditions, management practices and genotypes. Coefficients describing the normalized bell-shape function were used to quantify changes in the area-per-leaf profile. Our analysis revealed that only those coefficients that describe the breadth of the bell-shape function and the position of the largest leaf were altered by year, hybrid, plant density, nitrogen, and row spacing.

The effect of year on values of b and x_o may be related to incident solar radiation (PAR) and temperature during early phases of development. Values of b were inversely associated with the amount of incident PAR accumulated during a period of approximately 30 days after planting (Fig. 4.3a), indicating that area-per-leaf profile becomes flatter as incident solar radiation increases. Values of x_o were associated with mean temperature during a period of approximately 30 days after planting (Fig. 2.3b) in a quadratic fashion with an optimum temperature (i.e., the temperature in which the position of the largest leaf is the greatest) around 16 °C (Fig 2.3b). The effect of plant density on the breath of the area-per-leaf profile may reflect a response to the stress. In fact, the area-per-leaf profile was flatter in low than in high plant density, similar to the response to accumulated PAR. The lower position of x_o as plant density increased may be associated with a lower number of leaves and also would constitute an indication that competition for assimilates is initiated earlier at high plant density. In addition, the small changes in b and x_o with a reduction in row spacing suggest that a more uniform plant stand is not effective in alleviating early competition among plants. Thus, advantages in grain yield reported for narrow row maize could be attributed more to changes in light

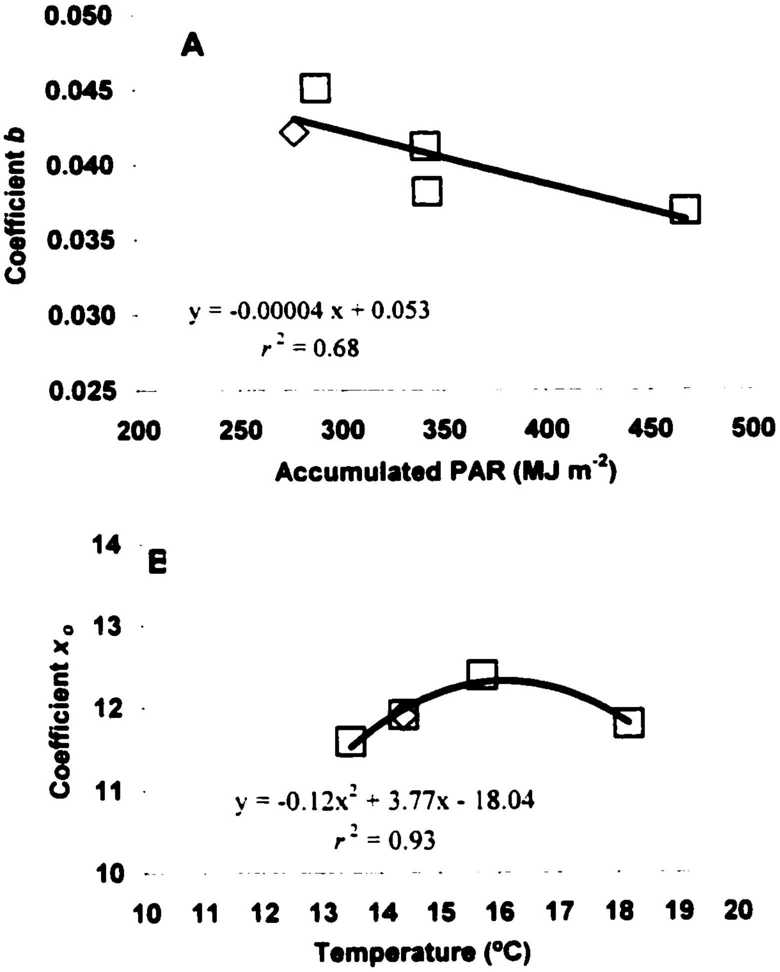

Fig. 4.3. Relationship between coefficent b and accumulated PAR (A) and between coefficient x_o and mean temperature (B) during a period of 30 days after planting. Data included four years data in Woodstock (squares) and one year in Elora (diamond).

distribution, total leaf area and/or leaf senescence than changes in the area-per-leaf profile. Flénet et al. (1996) reported a reduction in the extinction coefficient (i.e., a more uniform light distribution across the canopy) as row spacing increased from 0.35 to 1 m.

Breadth of the area-per-leaf profile seems to be a genotype specific trait and maximum differences among hybrids in coefficient *b* ranged from 13% (Data Set 3) to 19% (Data Set 4). Interestingly, the range of *b* values observed in Pioneer 3893, the hybrid present in all our data sets, included values reported by Dwyer and Stewart (1986b) and values estimated from the relationship reported by Keating and Wafula (1992) for a tropical plant with 19 leaves.

In spite of a flatter area-per-leaf profile due to increased PAR, low plant density and newer hybrids, the effect of the *b* coefficient on total leaf area as estimated from the normalized bell-shape function, was never greater than 10%. This percentage would represent the *per se* impact of changes in the breadth of the profile whenever the area of the largest leaf was comparable. In addition, the increase on total leaf area attributable to a higher placement of the largest leaf is negligibly.

Based on the relatively small impact of a wide range of conditions studied on coefficients describing the area per leaf profile, we were able to use a general equation to estimate areas of individual leaf from the area of the largest leaf and compare them with an independent data set. Although estimated values of individual leaf area were lower than observed values, the underestimation was consistent across different management practices and hybrids. Therefore, one can speculate that by quantifying the changes in the area of largest leaf associated with experimental year, diverse management practices or genotype, their impact on full leaf area could be easily determined.

Because of the robustness of the bell-shape function and the small impact of its coefficients on the full leaf area, we were able to modify the model MAIS by including our general equation. Observed values of individual leaves were in good agreement with those simulated by the modified model. However, the level of agreement varied with plant density. The fit was excellent for the low plant density (3.5 plant m^{-2}) but values fell below 1:1 line for the high plant density (12 plant m^{-2}). Possible reasons for the relatively poor agreement at high plant density could be related to the effect of an altered quality of light as plant density increases (Kasperbauer and Karlen, 1994). This aspect is not considered in the model and quantification of this effect could improve the predictive value of the model. Since the bell-shaped function requires the area of the largest leaf to calculate the total leaf area per plant, the effect of stresses on plant leaf area could be predicted when the effect of stress of the largest leaf has been quantified. Thus, by using this simple relationship, the use of the model MAIS could be extended to specific stress conditions, such us water and nutrient limitations.

In summary, this study supports the contention that the bell-shaped function is a robust predictor of the area-per-leaf profile in maize. A single general equation was proposed to estimate the area per leaf in a model that simulates maize growth and yield. Since the calculation of the profile depends on knowing the area of the largest leaf, we postulate that by measuring the impact of varying environmental or management conditions on the largest leaf, their respective effects on total leaf area could be determined. Even though two out the four coefficients of the function varied with year and agronomic practices, their *per se* impact on the total leaf area estimated from the area of the largest leaf was never greater than 10 %.

CHAPTER V

GENERAL DISCUSION AND CONCLUSIONS

GENERAL DISCUSION AND CONCLUSIONS

Since future yield improvement of maize in temperate regions relies predominantly on an increase in crop dry matter accumulation during the growing season, the role of green leaf area and leaf senescence in maintaining high rates of photosynthesis, in particular, throughout the grain-filling period is important. A robust and simple characterization of leaf area along with a better understanding of leaf senescence will be useful in maize breeding programs, particularly for those programs where enhanced dry matter accumulation during the grain-filling period is a breeding target. In addition, leaf area and its loss through leaf senescence are two essential components part of models that simulate crop growth and grain yield, and better estimations of factors associated with the formation and decline of leaf area across a wide range of conditions and genotypes are needed.

In this study, a comparison between an older and two more recent maize hybrids were made in terms of visual symptoms of leaf senescence. Functional symptoms of leaf senescence were examined in both groups of hybrids based on seasonal and diurnal changes of photosynthesis. In addition, the area-per-leaf profile was examined on several maize hybrids grown at various environments and agronomic practices.

In terms of visual senescence, the widely reported *stay green* characteristic of newer hybrids is determined by a greater green leaf area index at silking, a delay in the onset of leaf senescence and a lower rate of leaf senescence during the grain-filling period than that the older hybrid. However, accelerated leaf senescence during the grain-filling period in newer hybrids when grown at high plant density seems to be the result of a low source:sink ratio associated with a greater ability to set kernels around silking. In terms of functional senescence, newer hybrids showed a greater ability to sustain high leaf photosynthesis and a high efficiency of photosystem II during the grain filling period. Older and newer hybrids differed in functional symptoms of leaf senescence even though the loss in greenness did not produce these differences. This finding clearly demonstrates that differences in dry matter accumulation during the grain-filling period are not fully attributable to losses of green area and that leaf greenness *per se* is not sufficient to maximize carbon gain during the grain-filling period. In addition, in high grain yield environments newer hybrids tended to show a profile of leaf senescence where top and bottom sections of the canopy senesced earlier than the central section. Leaves close to the topmost ear remained healthier and greener than their counterparts in the top and bottom, which may represent an ideal type of plant for leaf senescence and grain yield. This study also showed a reduction of leaf photosynthesis during afternoon hours, though the magnitude of this reduction was not associated with leaf senescence and no differences were detected among hybrids. Although the nature of the experiments reported here did not allow us to identify the cause of seasonal and daily decline in photosynthesis, results of this study indicate that a decreased ability to dissipate the energy absorbed during the day should not be ruled out.

This study confirmed that the bell-shaped function is a robust predictor of the area-per-leaf profile in maize. When normalized to the largest leaf, the area-per-leaf profile showed only small changes in response to growing seasons, agronomic practices and genotypes. Consequently, the total leaf area per plant (and leaf area index) could be estimated quite reliably from the area of the largest leaf. In addition, a more expedite and quantitative score of the leaf senescence progress could be performed if the number of dead leaves were recorded.

In conclusion, results of this study confirm that newer hybrids have a greater LAI, delayed appearance of visual symptoms of leaf senescence, and sustained photosynthesis during the grain-filling period. Thus, *stay green* is not always coordinated with leaf photosynthesis, and these two traits should be tightly associated whenever increased dry matter accumulation during the grain-filling period is a breeding objective. In addition, this study demonstrated that the area-per-leaf profile constitutes a conservative characteristic that may be exploited to expedite estimations of leaf area and senescence in maize.

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Appendix 1. Probability values of sources of variance (ANOVA: split plot design) for variables leaf area index (LAI), dry matter accumulated at silking (DMS), total dry matter accumulated (TDM), grain yield (GY), harvest index (HI), number of ear per plant (EP), changes in stover weight (▲STW), relative rate of leaf senescence during first (RRLS1) and second (RRLS2) half of grain filling period.

Source of variation	LAI †	DMS	TDM	GY	HI	EP	▲STW	RRLS1	RRLS2
	-------------------	-------------------	-------------------	-------------------	P-value	-------------------	-------------------	-------------------	-------------------
Plant density (D)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0059
Maize hybrid (H)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Year (Y)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
D X H	0.3806	0.1930	0.0154	0.0155	0.0598	0.0001	0.0022	0.1675	0.0001
D X Y	0.0001	0.0001	0.0050	0.0005	0.6934	0.0343	0.0001	0.0001	0.0001
H X Y	0.0197	0.0169	0.0071	0.0001	0.0001	0.0219	0.0312	0.0001	0.0001
D X H X Y	0.2153	0.4356	0.2132	0.0043	0.0853	0.0002	0.7943	0.5449	0.3671
Model CV (%)	13.1	1.7	8.3	4.1	7.5	16.1	15.8	17.6	12.9

† ANOVA was performed using transformed variables: natural logarithm (LAI, DMS, TDM, GY, EP), root square (RRLS1, RRLS2), arc sin (HI), and 100 + (▲STW).

Appendix 2. Probability values of sources of variance (ANOVA: split plot design) for variables leaf area index (LAI), dry matter accumulated at silking (DMS), total dry matter accumulated (TDM), grain yield (GY), harvest index (HI), number of ear per plant (EP), changes in stover weight (▲STW), relative rate of leaf senescence during first (RRLS1) and second (RRLS2) half of grain filling period in 1999.

Source of variation	LAI †	DMS	TDM	GY	HI	EP	▲STW	RRLS1	RRLS2
	----------	----------	----------	----------	P-value	----------	----------	----------	----------
Plant density (D)	0.0001	0.0001	0.0001	0.0001	0.3210	0.0001	0.0262	0.0038	0.0002
Maize hybrid (H)	0.0001	0.3384	0.0001	0.0001	0.0003	0.0001	0.0097	0.0001	0.0001
D X H	0.0215	0.4745	0.0001	0.2549	0.7876	0.0001	0.1840	0.3237	0.0001
Model CV (%)	10.8	1.2	0.9	3.2	10.4	18.0	15.1	22.9	9.2

† ANOVA was performed using transformed variables: natural logarithm (LAI, DMS, TDM, GY, EP), root square (RRLS1, RRLS2), arc sin (HI), and 100 + (▲STW).

Appendix 3. Probability values of sources of variance (ANOVA: split plot design) for variables leaf area index (LAI), dry matter accumulated at silking (DMS), total dry matter accumulated (TDM), grain yield (GY), harvest index (HI), number of ear per plant (EP), changes in stover weight (▲STW), relative rate of leaf senescence during first (RRLS1) and second (RRLS2) half of grain filling period in 2000.

Source of variation	LAI †	DMS	TDM	GY	HI	EP	▲STW	RRLS1	RRLS2
	P-value								
Plant density (D)	0.0001	0.0001	0.0001	0.0009	0.0009	0.0002	0.0044	0.0001	0.0045
Maize hybrid (H)	0.0001	0.0195	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
D X H	0.2403	0.0812	0.7069	0.0030	0.0029	0.1319	0.0257	0.7862	0.0036
Model CV (%)	10.7	1.5	2.1	3.8	4.5	20.4	16.1	19.0	13.5

† ANOVA was performed using transformed variables: natural logarithm (LAI, DMS, TDM, GY, EP), root square (RRLS1, RRLS2), arc sin (HI), and 100 + (▲STW).

Appendix 4. Probability values of sources of variance (ANOVA: split plot design) for variables leaf area index (LAI), dry matter accumulated at silking (DMS), total dry matter accumulated (TDM), grain yield (GY), harvest index (HI), number of ear per plant (EP), changes in stover weight (▲STW), relative rate of leaf senescence during first (RRLS1) and second (RRLS2) half of grain filling period in 2001.

Source of variation	LAI †	DMS	TDM	GY	HI	EP	▲STW	RRLS1	RRLS2
	------------	------------	------------	------------	P-value	------------	------------	------------	------------
Plant density (D)	0.0001	0.0001	0.0001	0.0008	0.0012	0.0001	0.0024	0.0001	0.0259
Maize hybrid (H)	0.0001	0.0323	0.0001	0.0001	0.0145	0.0001	0.0001	0.0001	0.0001
D X H	0.3212	0.3552	0.0070	0.0001	0.0001	0.0001	0.1501	0.1538	0.0391
Model CV (%)	14.3	1.8	1.4	2.3	3.5	15.8	16.7	15.1	14.8

† ANOVA was performed using transformed variables: natural logarithm (LAI, DMS, TDM, GY, EP), root square (RRLS1, RRLS2), arc sin (HI), and 100 + (▲STW)

Appendix 5. Probability values of sources of variance (ANOVA: split plot design) for variables carbon leaf exchange (CER), stomatal conductance (COND) and Fv/Fm ratio.

Source of variation	CER	COND	Fv/Fm
	---------------------------P-value---------------------------		
Light (L)	0.0001	0.0001	0.0016
Year (Y)	0.0001	0.0001	0.0001
Stage (S)	0.0001	0.0001	0.0001
Time (T)	0.0001	0.0001	0.0001
Hybrid (H)	0.0001	0.0036	0.0021
L X Y	0.1394	0.2142	0.3172
L X S	0.0001	0.0058	0.9263
L X T	0.1127	0.3201	0.0499
L X H	0.0077	0.0814	0.3918
Y X S	0.0003	0.5081	0.0001
Y X T	0.2833	0.0680	0.0001
Y X H	0.4371	0.2276	0.2400
S X T	0.0403	0.0040	0.0277
S X H	0.0003	0.3224	0.0023
T X H	0.1633	0.4113	0.9582
L X Y X S	0.6489	0.0694	0.6069
L X Y X T	0.6693	0.3198	0.1613
L X Y X H	0.9087	0.3345	0.1002
L X S X T	0.9609	0.7194	0.6239
L X S X H	0.9026	0.1279	0.5543
L X T X H	0.6236	0.6952	0.6906
Y X S X T	0.1966	0.3667	0.0717
Y X S X H	0.7755	0.8045	0.2295
Y X T X H	0.7678	0.9970	0.8594
S X T X H	0.7055	0.2396	0.9554

Source of variation	CER	COND	Fv/Fm
	---------------------------P-value	---------------------------	
L X Y X S X T	0.5577	0.6798	0.7071
L X Y X T X H	0.2738	0.5762	0.1876
L X S X T X H	0.5023	0.4790	0.6666
L X Y X S X T X H	0.7570	0.4694	0.7982
Model CV (%)	15.1	27.2	4.2