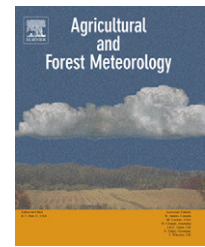


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Estimation, variation and importance of leaf curvature in *Zea mays* hybrids

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ARTICLE INFO

Article history:

Received 5 November 2007

Received in revised form

13 May 2008

Accepted 14 May 2008

Keywords:

Zea mays

Leaf angle

Leaf auricle

Leaf curvature

Leaf midrib

Leaf morphology

ABSTRACT

Maize leaves are curved but this curvature cannot be described by a single system of equations based on conic sections. We made comparisons of curvature between hybrids, leaf positions and planting density using measures of maximum leaf height and leaf tip:maximum leaf height ratio. With these measures a modern hybrid released in 1991 (Pioneer 3394) shows less curvature compared with hybrids released in 1963 (Pioneer 3306) and 1936 (Pioneer 307). The greater uprightiness of 3394's leaves was apparent for all leaves measured. Curvature is a plastic character. While all hybrids developed less curvature in higher density plantings the effect was greatest for 3394. 3394 had shorter, more lanceolate leaves with smaller leaf area, a lower estimated torque around the point of leaf attachment to the stem, and a significantly smaller auricle base and smaller angle between the leaf blade and auricle base. These results support suggestions that the pattern of curvature may be produced by the progression of irreversible bending during leaf development. We found no consistent relationships between differences in curvature and measurements of leaf midrib morphology or anatomy.

A frequently used approach in modeling foliage area distribution by angle is to use an approximation provided by the angular distribution of an ellipsoid. We show that for leaves in this study this approach is biased and that a Richards function describes the cumulative frequency distribution of leaf area by inclination angle more effectively. The importance of an unbiased estimator is discussed in relation to the variation in photon flux density received by surfaces of different inclinations.

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1. Introduction

The potential for increasing crop yields by manipulating leaf angle has attracted attention over decades. Pendleton et al. (1968) made leaves of a popular 1960s maize hybrid, Pioneer 3306 (a single cross hybrid commercially released in 1963)

more upright by tying them at an angle of 10° declination from the stalk from pollination through to harvest. The plants were grown at 59,304 plants ha⁻¹ and when all leaves were tied yield was 6.5% greater than the untied control; when only leaves above the ear were tied yield was 14.2% greater suggesting the importance of maintaining high light inter-

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doi:10.1016/j.agrformet.2008.05.015

ception by the canopy as a whole if leaf angle is to be manipulated. Winter and Ohlrogge (1973) repeated these manipulations of leaf declination with three other hybrids grown at different densities, and report that obtaining an increased yield with more vertical leaves requires a leaf area index (L) of 5 or greater—see also Pepper et al. (1977). Yield trials of maize hybrids released from the 1930s to the 1990s show a continuous increase in yield that is greater at higher planting density, and one of the positive correlates with this increased yield is an increase in leaf angle score (Duvick et al., 2004).

Both theoretical models, and those based on computer simulation, have been used to analyze and discuss possible effects of leaf angle on maize crop yield. Among theoretical approaches upright leaves above the ear and more horizontal leaves below it have become a recurring component of suggestions for increasing productivity and development of maize ideotypes (e.g., Duncan, 1971; Mock and Pearce, 1975; Hawkins, 1982; Long et al., 2006). However, not all investigators agree on the importance of leaf angle. Monteith (1969) suggests that for L between 4 and 8 crop photosynthesis is not strongly dependent upon leaf angle and Sinclair and Muchow (1999) report that model-based analyses indicate leaf angle does not affect radiation use efficiency. Stewart et al. (2003) conclude that depending on row widths, maize hybrids with very upright leaves can have both the smallest and largest, daily canopy photosynthesis.

Potentially, modeling can be an important tool for analyzing the influence of leaf angle and its interaction with leaf area index (L) on yield. Generally maize canopies have been modeled as horizontal layers (e.g., Lizaso et al., 2005) or three-dimensional zones (e.g., Stewart et al., 2003). The frequency distribution of foliage area by leaf angle has been approximated for each layer or zone using empirical frequency distributions, or by describing distribution of leaf area by angle using the angular distribution of surface area on an ellipsoid—see Monteith (1973, p 39–58) for principles; Campbell (1986, 1999) for calculations; Lizaso et al. (2005) for use in a canopy model.

However, maize leaves are curved and this complicates how foliage angle may be expressed. In recent developments models of curvature for the individual leaf have been incorporated into individual plant-based growth models (e.g., España et al., 1999). To use modeling to resolve the question of whether differences in curvature may contribute to differences in yield between hybrids, it is important that descriptions of curvature are used that define differences between hybrids.

Curvature of maize leaves is the result of a complex set of processes. While classical mechanics has provided a starting point for modeling (Niklas, 1992, p. 133) its equations apply to loaded beams with small deflections and Moulia et al. (1994) report that using them requires smoother curves than they found along maize leaves and that leaf deflection is affected by shear forces, as well as elasticity. Moulia and Fournier (1997) developed a mechanical model for the midrib of maize as a composite beam comprising the midrib cortex and the abaxial vascular bundles embedded in sclerenchyma, which they call the “sclerified rind,” and find that 95% of the flexural stiffness is in this “rind.” Hay et al. (2000), working with maize plants that had developed their lowermost 5 or lowermost 7 leaves

(V5 and V7 stages, respectively, Ritchie et al., 1993) suggest that elastic bending of leaves under self-weight is relatively unimportant and that curvature is the result of elastic deformations under weight during development. However, from analysis of mutant phenotypes, they also suggest it is affected by midrib structure.

In this work we ask: How can leaf curvature be defined and does it vary among selected hybrids? How might variation in leaf curvature be produced? How can the relationship between leaf area and its angle of inclination be represented? In previous work with seedlings of Pioneer 307 (a double cross hybrid commercially released in 1936), Pioneer 3306 (used by Pendleton et al., 1968), and Pioneer 3394 (a single cross hybrid released 1991 and extensively used through the mid-west of the USA in the 1990s), juvenile leaves (*sensu* Freeling, 1992) of 3394 were more upright than those of the two older hybrids (Fellner et al., 2003, 2006). Here we characterize differences in curvature between non-juvenile leaves, with leaf numbers seven or greater, for the same three contrasting hybrids and describe concomitant differences in leaf area, length and weight. For two hybrids, 3306 and 3394 we describe the effects of planting density on these leaf characters and investigate anatomical and morphological features that might contribute to differences in curvature. We investigate differences between hybrids in the dimensions of leaf auricles, i.e., the tissue on either side of the midrib in the position of a “hinge” between leaf sheath and leaf blade, because preliminary observations with seedlings suggested auricle growth might contribute to observed differences in leaf curvature. We discuss whether the reduced leaf weight found in leaves of 3394 might contribute to development of upright leaves. Given the range of variation in curvature we report, and the causes that may underlie it, we examine the suitability of some current approaches in modeling curvature and the relationship between leaf area and leaf angle.

We report results from three experiments: (1) measurements of leaf curvature, dimensions, weight and area of all three hybrids grown in a greenhouse where plants would be free from physical damage. (2) A field comparison for plants of 3306 and 3394 each grown at two initial planting densities and for which we also measured aspects of midrib morphology and anatomy. (3) A greenhouse experiment with all three hybrids where we measured leaf curvature and auricle dimensions.

2. Materials and methods

2.1. Experiment 1

Kernels from Pioneer hybrids 307, 3306 and 3394 (Pioneer HiBred Intl; Des Moines, IA, USA) were planted in soil (Sunshine no. 4 mix; Sun Gro Horticulture, Bellevue, WA, USA) in pots (22.9-mm high, 241-mm diameter, 1 seed per pot) in a greenhouse. Smith et al. (2004) give pedigree information on these hybrids. The plants were inspected daily, regularly watered and weekly fertilized with a balanced nutrient mix (Plant Food; Miracle-Gro, Port Washington, NY, USA) in the irrigation.

A power analysis based on greenhouse grown plants and using leaf length, weight and area indicated that six replicates

were adequate for detecting differences between leaf position and hybrids in these quantities and that, for leaf curvature measurements of leaf angle should be made at 50 rather than 100-mm intervals. Consequently 6 replicates of each hybrid were used and the 18 pots were randomized and arranged in 3 rows of 6, separated by 800 mm, and positions on the bench were rotated twice weekly, to reduce any effect of variation in the greenhouse environment, but the azimuth orientation of pots remained the same. The first leaf produced was labeled 1 and leaves 7 through 10 were measured after 7 weeks growth when leaf and internode elongation had ceased. At this stage plants were actively growing and not pot bound but were reaching a maximum size for greenhouse conditions. Leaves were measured and harvested in the sequence 7, 8, 9, and 10 at 2-day intervals with all leaves of the same number for all three hybrids being measured in the same 2-day period.

Leaf declination from the stem was measured on intact plants by first marking 50-mm increments along the underside of the midrib and then holding a protractor with swinging needle at its origin along the midrib of the section. Recording declination from the vertical rather than inclination from the horizontal has the advantage of distinguishing leaf sections on different sides of the horizontal point that occurs on many maize leaves. Tests showed negligible differences between operators using this method. Area and dry weight were measured for each section on leaves 7 and 9. Area was measured with a LI-3100C Area Meter (LI-COR Biosciences, Lincoln, NE, USA) and dry weight after drying at 70 °C for at least 48 h. Leaves in envelopes were cooled to room temperature for 15 min and weighed on a Mettler H80 to four significant places.

2.2. Experiment 2

Hybrids 3306 and 3394 were planted in late May 2000 at the Pioneer Hi-Bred International, Inc. experiment farm, Johnston, Iowa, in rows 762 mm (30 in.) apart, following usual commercial practice. Two spacings along the rows were used: 137 mm, giving 11,609 plants ha⁻¹ subsequently referred to as LD for Low Density, and 113.7 mm giving 95,095 plants ha⁻¹ subsequently referred to as HD for High Density. On 17–20 July when leaves 11, 12, 13 and 14 were fully formed but had not started to senesce, leaf declination of each successive 50-mm section was measured on six plants in each combination of 2 (hybrids) × 2 (planting densities). At this stage of growth, i.e., anthesis, the maize canopy is at its maximum size. Leaves 11 through 14 are the largest in the canopy and, typically, the ear forms in this region of the stem. All samples were taken from one central row in a block of plants of the same hybrid and spacing. Typically, at the HD spacing, a single ear forms in the axil of leaf 12 while at LD multiple ears formed at leaves 11, 12 and 13.

Measurements of curvature were made as described for Experiment 1. Subsequently, leaves were cut from the stem with a scalpel at the ligule, placed in a plastic bag, sealed, and transported overnight in a cooler with blue ice to the University of Washington. Area and weight were measured for leaves 11 and 13 using the method described for Experiment 1.

2.2.1. Midrib morphology and anatomy

For leaves 12 and 14, 1-cm segments of complete midrib and connecting lamina were removed at 5 and 45 cm along the leaf

from point of attachment with the stem and stored in methyl alcohol. These samples were progressed through an eight-step dehydration sequence to 100% butanol and then embedded in paraffin (Paraplast 56 °C melting point) and mounted on wood blocks (Sass, 1958). 35- μ m sections were cut using a sledge microtome, and then adhered to a microscope slide using a hot plate. Slides were immersed in successive Coplin staining jars containing toluene (10 min), ethanol (2 min) and four increasing water in ethanol concentrations to 50% ethanol–water (2 min) and stained with safranin–fast green in 50% ethanol–water for 30 min. Slides were then immersed in three successive Coplin staining jars containing increasing ethanol concentrations to 95% ethanol–water followed by fast green for 30–60 s with younger leaf samples receiving the longer time. After washing the slides in ethanol followed by toluene cover slips were placed over the sections using Permount and slides were dried at 40 °C.

Images of the complete midrib were obtained using the Scion Image software (Scion Corporation, MD, USA) for microscopy. The distances between abaxial and adaxial epidermis were measured at 0.1 mm and area and moment of area were calculated from the data using a computer program. Distances (a) between veins, (b) from epidermis across the sclerenchyma tissue to the adaxial tip of the vein in the midrib cortex, and (c) from the abaxial surface of the leaf to the interior apex of xylem bundles, were measured under the microscope with a calibrated graticule.

2.3. Experiment 3

Eight plants of hybrids 307, 3306 and 3394 were planted in a greenhouse as described for Experiment 1. Initial measurements of auricles suggested an increase in replicates from six to eight was required to investigate for differences between hybrids and leaves but plants were not grown to the same size as in Experiment 1 and so leaves lower on the plant were investigated. Pots were similarly randomized, arranged and their positions on the bench changed and leaves were numbered in the same way. Leaf declination for successive 5-cm lengths from the stem were measured for leaves 5, 6, 7, and 8 of each plant as described for Experiment 1. Leaf 8 was not fully developed at the time of measurement and was deliberately selected to obtain indication of the ligule development process. Each leaf has two auricles, pale green, wedge-shaped tissues at each side of the leaf–sheath junction (Sharman, 1942). Auricle cells are formed and they divide as the blade and sheath expand and enlarge at the time of outgrowth of the ligule (Osmont et al., 2003). After the leaf emerges auricle cells expand further allowing the leaf blade to bend out from the main axis. We refer to the length of the auricle along the base of the leaf as the top, its length along the sheath as the bottom (Fig. 1). These sides join at their connection with the midrib and we refer to the length that completes the triangle as the side. These dimensions were measured for both triangles of tissue on each side of the leaf and we report mean values for these two triangles.

2.4. Calculations

Calculations were made using S-Plus (Insightful, Corp., Seattle) and R 2.6.2 (Comprehensive R Archive Network

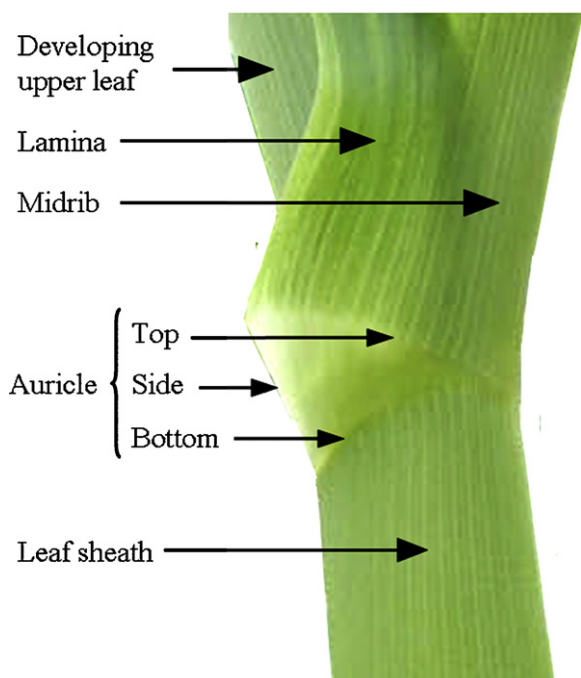


Fig. 1 – An auricle is a triangular-shaped tissue at the junction between leaf and leaf sheath. Each leaf has a pair of auricles, one on each side of the stem, with their sides facing in the same direction. One auricle of a leaf is shown and the measured lengths of auricle top, bottom and side are indicated. The abaxial side of the labeled leaf and the midrib are to the right. The lamina, towards the left, first extends vertically upwards from the auricles then curves and flattens to lie on either side of the midrib as the leaf curves away from the stem. A second, younger, leaf can be seen emerging above the labeled leaf.

2008). For the Experiment 1 investigation of variation in leaf shape the function *smooth.spline* was used to fit a natural cubic spline with a knot placed at every data point and the *predict* function used on the *smooth.spline* object to produce first and second derivatives. For Experiment 2, where parsimonious models were sought, *ns* was used for natural cubic splines and *bs* for polynomial splines (Chambers and Hastie, 1992, Chapter 7) and model selection was made using the Akaike Information criteria (AIC), function *AIC*. Solutions for non-linear regression equations and the Richards function used the *nls* function (Chambers and Hastie, 1992, Chapter 10). Analysis of variance follows procedures defined by Zar (1999) and with multiple comparisons made using the Tukey test. The ANOVA for leaf tip:leaf maximum height, which is a proportion, was made on arcsine (square root) transformed data (Zar, 1999: p. 278).

3. Results

3.1. Experiment 1

Vertical profiles of the leaf midrib were calculated from successive 50-mm leaf-section declinations using Cartesian

geometry, with *x* the horizontal distance from the point of attachment of the leaf to the stem, and *y* the vertical distance. Our objective was to compare hybrids and we sought a system of equations that could describe curvature of the complete leaf for all hybrid \times leaf position combinations. Comparison between hybrids could then be made by examining parameter values. For example, Stewart and Dwyer (1993) developed a quadratic equation for maize-leaf curvature and Antunes et al. (2001) provide a modification of Stewart and Dwyer’s 1993 approach to include leaves with no downturn in curvature in their more distal part. Prévot et al. (1991) model the ascendant part of the leaf, as defined by the central vein, as an arc of a parabola, and the descendant part as an arc of an ellipse.

We found two types of variation in curvature prevented the use of conic sections, e.g., sections of circles, parabolas or ellipses, all of which have quadratic terms. First there was substantial difference between leaves in morphology distal to the point of maximum leaf height (Fig. 2). In some instances the descendent part was recurved, i.e., where the tip is closer to the stem than parts of the leaf proximal to it as for the lower leaf in Fig. 2. In many cases this recurvature was not smoothly consistent with its more proximal sections. Second, there are distinct changes in the shape of the curvature along the leaf before, as well as after, the point of maximum height. This is illustrated by fitting a cubic smoothing spline, using *smooth.spline* in R, and then operating on the *smooth.spline* object with the *predict* function to calculate first and second derivatives of leaf shape (Fig. 3). Generally the cubic smoothing spline provides a close fit to the leaf shape except for the distal part of leaves when a leaf is recurved towards the stem, as for the 3306 leaf shown in Fig. 2. The point of maximum height, where the first derivative is zero, is further along the leaf for 3394 > 3306 > 307. In the ascendant part of the leaf there are distinct changes in the second derivative, which for the 307 and 3306 examples (Fig. 3) first decreases then increases, while for the 3394 example there are three values for proximal positions that are markedly lower than the subsequent

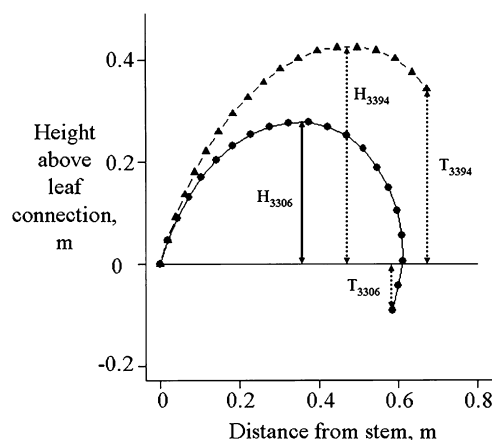


Fig. 2 – Illustration of metrics used in comparison of leaf curvature. This example is for leaf 8 midrib profiles of greenhouse-grown hybrids 3306 and 3394. Maximum height, *H*, is the highest point reached above the point of attachment of leaf to stem. $H_{3306} = 27.7$ cm, $H_{3394} = 42.6$; the tip/height ratios, *T:H*, are, respectively -0.33 and 0.81 for 3306 and 3394.

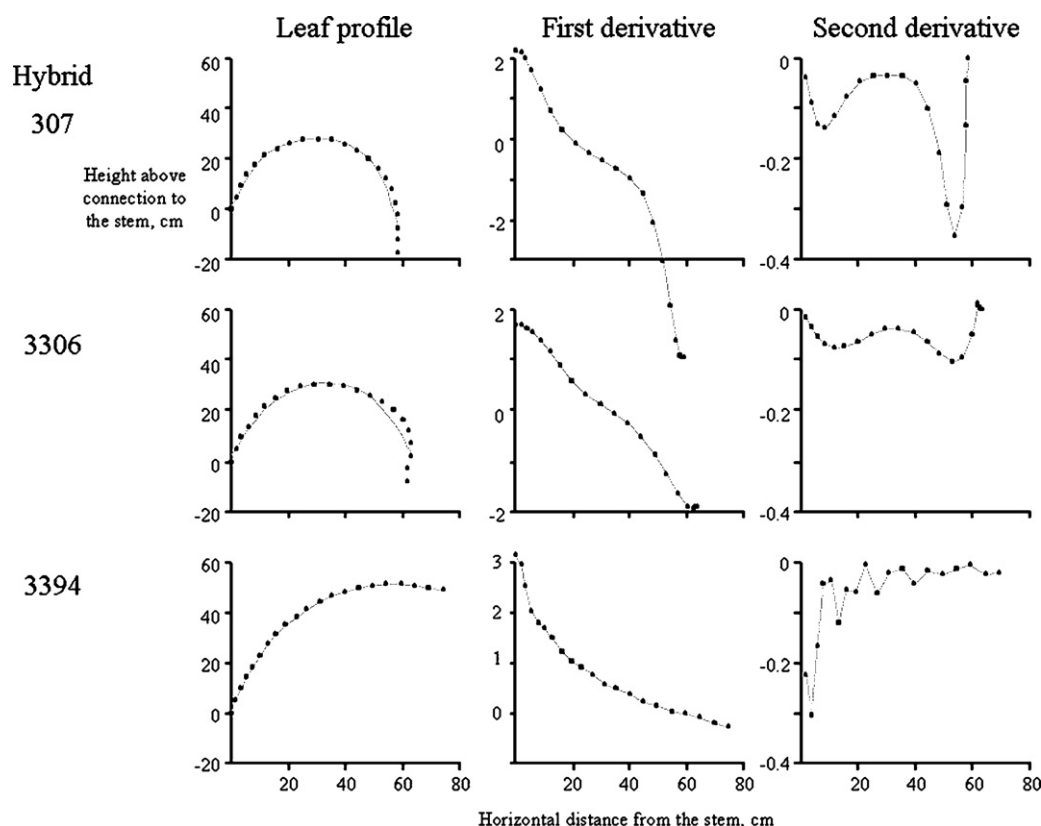


Fig. 3 – Examples of leaf profiles, left column, of each hybrid with fitted cubic splines. Center column is the first derivative of the curvature and right column the second derivative both calculated from the fitted cubic splines.

gradual increase. These derivatives were calculated for all leaves in this experiment and Experiments 2 and 3. The profiles illustrated in Fig. 3 are typical for each hybrid but within each hybrid \times leaf position there was variation in the pattern of derivatives both proximal and distal to the inflexion point. This variation resulted either in failure in fitting a non-linear regression using quadratic terms, or else markedly correlated residuals at successive positions along some section of the leaf. Examples of variation within leaf categories is discussed in the results for Experiment 2.

As these were greenhouse-grown plants we conclude that the complexity in leaf curvature is an intrinsic property of maize leaves at least for these hybrids. Profiles of the radius of curvature (Silk and Erickson, 1978) in 5-cm sections along each leaf are complex functions of distance from the blade joint (e.g., Hay et al., 2000) that cannot be used to characterize differences between hybrids. For initial comparisons of

curvature we use the empirical values of maximum height of leaf (H) and tip:maximum height ratios ($T:H$).

Two-way ANOVAs, hybrid \times leaf position, were made for H , and $T:H$. Hybrid differences occur at $p < 0.001$, with 3394 having greater H and $T:H$ than either of the other hybrids with no significant difference between the older hybrids (Table 1). There are no interaction effects between hybrid and leaf position. Based on these metrics leaves of 3394 are more upright than those of 3306 and 307. There are also significant ($p < 0.01$) differences in H with leaf position although this effect is mainly due to a large gradation in hybrid 3394, for which mean values of H for leaves 7, 8, 9 and 10 are, respectively 39.88, 41.24, 45.31 and 47.51 cm. These results establish that H and $T:H$ can characterize leaves at different positions on the plant and define differences between hybrids.

A two-way ANOVA, hybrid \times leaf position of leaf area, shows 3394 with significantly ($p < 0.001$) shorter leaves and

Table 1 – Results of ANOVA between hybrids for leaf measurements for Experiment 1

Hybrid	H (cm)	$T:H$	Length (cm)	Area (cm ²)	Mass (g)	Torque (N m)
307	27.02a	-0.516a	106.95a	767.6a	2.39	0.006580a
3306	29.26a	-0.302a	108.12a	765.8a	2.62a	0.007164a
3394	43.49b	0.764b	101.67b	605.9b	2.42a	0.005141b

Differences between hybrids in H , $T:H$ (defined in Fig. 1) and leaf length. Within each column mean values with the same letter do not differ significantly between treatments at $p < 0.001$ on the basis of Tukey's multiple comparison test. Leaves at 4 positions, 7 through 10 were used for H , $T:H$ and length; leaves at positions 7 and 9 were used for area, weight and torque.

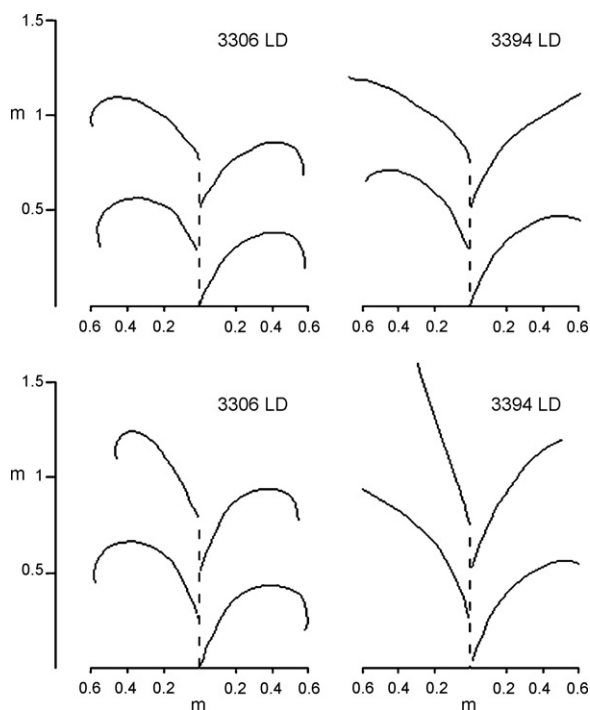


Fig. 4 – Vertical midrib profiles from measurements of leaf declination for 5-cm sections of individual leaves for each hybrid and planting density in the field. Leaves 11–14 (bottom to top) are shown. Leaves with median curvature were selected from within six replicates for each leaf position. To clarify comparisons of curvatures leaves are inserted at fixed 0.25-m heights in this figure.

smaller leaf area than the other two hybrids (Table 1). We also find significant differences ($p < 0.001$) in leaf area between leaf positions 7, mean area 575.1 cm² and 9, mean area 851.1 cm² but no significant hybrid \times leaf position interaction. There are no significant differences in leaf weight between hybrids but there is between leaf positions ($p < 0.001$), mean dry weight of leaf 7 was 1.896 g and leaf 9, 3.278 g, and no significant interaction between hybrid and leaf.

We calculated the torque, T (N m), exerted by the leaf at its point of attachment to the stem. $T = Fr$ where r = the horizontal distance from the point of attachment and $F = \text{mass} \times g$ (g is acceleration due to gravity). We calculated T by summing for successive 5-cm sections using leaf dry weight to represent mass. There are significant differences

($p < 0.001$) in torque between hybrids with $3394 < 307$ and 3306 but no differences between the two older hybrids (Table 1) and no significant interaction between hybrid and leaf position.

3.2. Experiment 2

3.2.1. Leaf morphology and weight

Leaves of 3394 are more upright than those of 3306, and in both hybrids the high density planting produced more upright leaves (Fig. 4). A three-way ANOVA, hybrid \times planting density \times leaf position, shows hybrid and planting density differences for both H and $T:H$ at $p < 0.001$ with $3394 > 3306$ and $HD > LD$ (Table 2). For both H and T there is significant ($p < 0.01$) hybrid \times planting density interaction with hybrid 3394 responding more to changes in planting density than 3306. For H there is a significant ($p < 0.05$) hybrid \times planting density \times leaf position effect with leaf 14 of 3394 having a greater increase in height at greater planting density with mean values: leaf 11, 56.14 cm; leaf 12, 61.81; leaf 13, 68.10; leaf 14, 78.46 but there were no other significant interactions in H or $T:H$. The greater maximum height found in the field experiment compared to the greenhouse for the same hybrids was expected because lower leaf numbers, 7–10, measured in the greenhouse, are generally smaller than mid-canopy leaves, particularly 11 through 13, measured in the field.

Within each replicate there is variation in the form of leaf curvature. Fig. 5 shows the results of model fitting for each replicate leaf 10 HD planting. AIC was used to define a parsimonious spline model of best fit. In all cases non-spline polynomial models (quadratic and cubic) were rejected in favor of spline models. For all six leaves of 3394 a second-order spline model was selected and the number of knots in the spline was 3 or 4. For leaves 1 through 5 of 3306 a cubic spline model was selected, but a second-order model for leaf 6, and the number of knots varies between 3 and 5. Interestingly a knot is required in the middle to lower section of the ascendant part of most leaves confirming the result from Experiment 1 that there are usually changes in curvature in this section.

A model with the same form cannot be solved for all leaves whether based on a conic section or on polynomial splines. However, curvature between the point of leaf attachment to the main stem and the maximum height of the leaf can be approximated for all leaves by an exponential saturation function:

Table 2 – Major effects from ANOVA between hybrids and planting density for leaf measurements for Experiment 2

	H (cm)	$T:H$	Length (cm)	Area (cm ²)	Mass (g)	Torque (N m)
Hybrid 3306	35.07e	0.288e	94.06c	922.4e	5.22a	0.010833e
Hybrid 3394	56.67f	0.940f	92.50d	833.8f	5.29	0.008696f
11,609 plants ha ⁻¹	38.35e	0.536e	90.31e	935.3e	6.40e	0.011870e
95,095 plants ha ⁻¹	53.39f	0.693f	96.25f	821.0f	4.10f	0.007658f

H and $T:H$ are defined in Fig. 1. Within each major effect, hybrids (within which planting densities are pooled) and planting density (within which hybrids are pooled), no significant difference at $p < 0.05$ between the pair of values is indicated by both values having the letter a. Significant difference between individuals at $p < 0.01$ is indicated by letters c and d and at $p < 0.001$ by e and f. Leaves at 4 positions, 11 through 14 were used for H , $T:H$ and length; leaves at positions 11 and 13 were used for area, weight and torque.

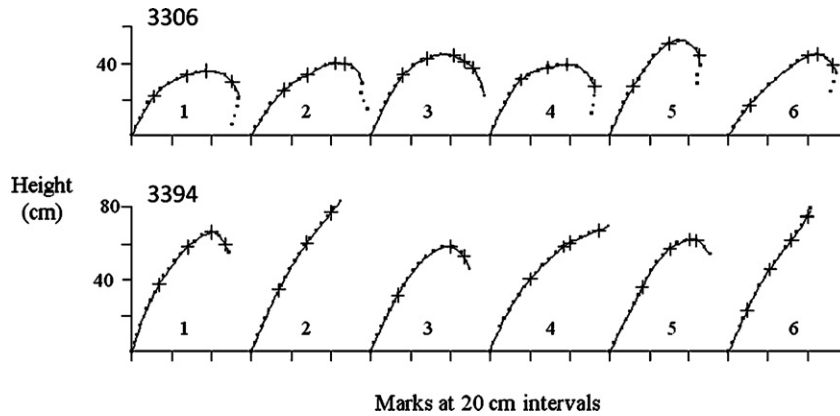


Fig. 5 – Profiles of replicates of leaf 10 from the HD treatment, Experiment 2: ●, data points; —, fitted spline model and +, position of knots in the spline model.

$$y = k_1(1 - e^{-k_2x}) \tag{1}$$

where k_1 scales the maximum value and k_2 determines the steepness of the curve. When Eq. (1) is fitted to the pooled replicates of each leaf of each hybrid and planting density combination residual sum of squares from the fitted curve is always <1%. This curvature is steeper for 3394 (Fig. 6) and for all leaves HD is steeper than LD. There were substantial differences in k_2 with less negative values indicating less curvature and more upright leaves. Mean values were: -0.0578 for hybrid 3306 at LD; -0.0513 for 3306 HD; -0.0406 for 3394 LD; -0.0340 for 3394 HD. ANOVA of k_2 based on fitting Eq. (1) to each individual leaf showed significant ($p < 0.001$) differences between hybrids and between planting density and the mean increase in k_2 for all leaves from low to high planting density was 11% for 3306 and 16% for 3394. Within 3394 HD there was a continuous decrease in curvature with increasing leaf number and leaf 11 had significantly greater curvature than leaf 14.

A three-way ANOVA, hybrid × planting density × leaf position, shows significant differences in leaf length between

planting densities with HD > LD and hybrid 3306 > 3394 (both $p < 0.001$) (Table 2). Leaf length decreases with increasing leaf number: for hybrids and planting densities combined mean lengths were: leaf 11, 95.83 cm; 12, 95.62; 13, 92.92; 14, 88.75 with a least significant difference of 5.66 ($p < 0.05$) so that leaf 14 is significantly shorter than leaves 11, 12 and 13 but there is no significant difference between those three leaf positions. There is no significant interaction between hybrid and planting density.

A three-way ANOVA of leaf area for leaves 11 and 13, hybrid × planting density × leaf position, shows highly significant differences between hybrids (3306 > 3394) and planting densities (LD > HD) (Table 2) but no significant difference between leaves 11 and 13. So although HD plants have more elongated leaves their areas are less than those of LD plants. ANOVA also shows significant ($p < 0.001$) difference in leaf weight, LD > HD (Table 2) but not between hybrids or leaf positions. There is significant interaction for leaf weight between planting density and leaf position with leaf 13 responding more than leaf 11 to LD, and between hybrid and leaf position again with leaf 13 having greater weight for 3306.

Profiles of area of 5-cm length sections along leaves show differences between hybrids and planting density that reflect the differences in area and length and illustrate changes in taper (Fig. 7). All leaves are tapered towards the tip and have proximal zones with constant, or initially increasing, area in successive 5-cm sections. For both hybrids area of sections in the proximal zones is greater at LD and within each planting density the area of 3306 > 3394. In the distal zone of the leaf where the leaf rapidly tapers, LD plants show a more rapid taper than HD plants and at both planting densities the taper for 3394 is more rapid than that of 3306. Second-degree polynomials are fitted to the profiles of area (Fig. 7):

$$y = k_1 + k_2x + k_3x^2 \tag{2}$$

where x is distance along the leaf and y is leaf area in successive 5-cm length sections, but with the most proximal 5-cm section not included in the model because it tended to have markedly greater area than other sections (Fig. 7). Parameter values for the fitted equations in Fig. 7 are given in Table 3. Generally leaves sampled in Experiment 1, which were from

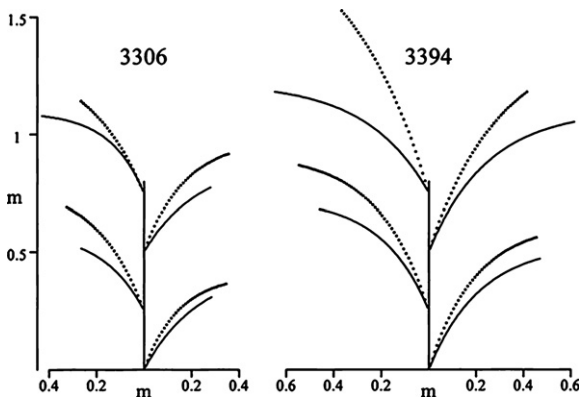


Fig. 6 – Effect of planting density on modeled leaf curvature. Each line obtained from parameters of Eq. (1) fit to the six replicates taken together for each hybrid and planting density combination as in Fig. 2. LD, continuous line; HD, dotted line. Data are shown for the ascending portion of the leaves only.

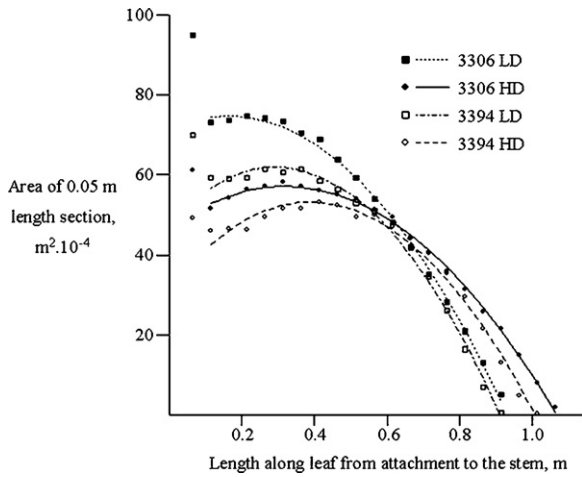


Fig. 7 – Profiles of leaf area in successive 5-cm increments along leaf 11 of hybrids 3306 and 3394 at LD and HD. For each hybrid and planting density combination a second-order polynomial is fitted to the mean data for six replicates.

lower on the plant, had a greater maximum at between one-third and half of leaf length from the stem attachment than those shown in Fig. 7 though the distribution was effectively fitted by a second-degree polynomial.

The distribution of leaf area at different angles can be calculated by combining differences in declination as leaves curve with differences in leaf area along the leaf. The frequency distribution of leaf area by declination shows general differences between hybrids and leaf position. A comparison of frequency distributions for leaf 13 at HD is shown in Fig. 8. 3306, like 3394, has its greatest individual percent of leaf area at low declination, i.e., >20–25° but those maximum percentages are less for 3306 than 3394 and there is a greater percentage of leaf area at higher declination angles (i.e., more horizontal) for 3306. A two-sample Kolmogorov–Smirnov test using the cumulative frequency distribution of the data represented in Fig. 8 showed the distributions to be significantly different ($p < 0.05$). Similar differences between hybrids were found for leaf 13 LD ($p < 0.01$) and leaf 11 HD ($p < 0.05$). The same general distribution shapes were seen for leaf 11 LD but were not significantly different ($p = 0.109$). For Experiment 1 the frequency distribution of leaf area by

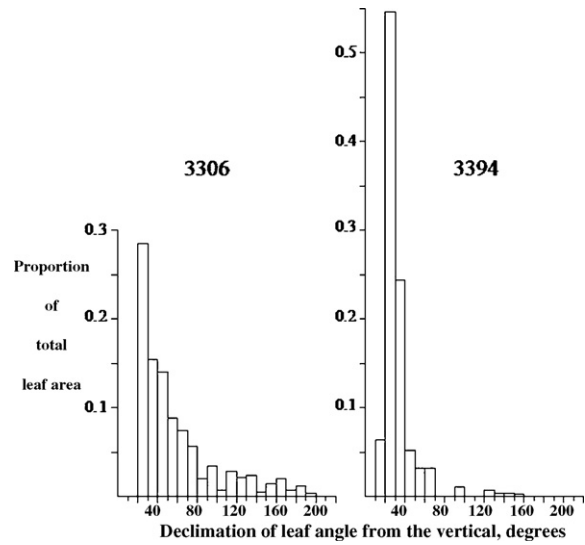


Fig. 8 – Frequency distribution of proportion of foliage area by declination angle of the adaxial leaf surface from the vertical for leaf 13 of hybrids 3306 and 3394 grown at 95,095 plants ha⁻¹. Each histogram is constructed from the 5-cm sections along six replicate leaves. 90° indicates a horizontal leaf section; angles >180° indicate recurved leaf tips.

declination is more left skewed for hybrid 3394 than the other two hybrids and for both leaves.

In simulation models of canopy photosynthesis, the interception of light has been approximated by assuming that the angular distribution of leaf area can be represented by the angular surface of an ellipsoid. For maize, which has a high proportion of upright leaf area, prolate ellipsoids are used for which the vertical axis is greater than the horizontal axis. The shape of the prolate ellipsoid is defined by $\chi = \text{horizontal axis} / \text{vertical axis}$. As examples (Fig. 9), we show the angular

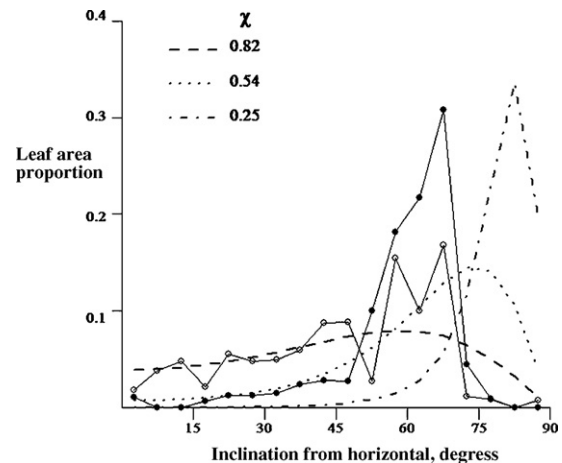


Fig. 9 – The proportion of surface area at successive angles of inclination from the horizontal on prolate ellipsoids with different values of χ (ratio of horizontal:vertical axis of an ellipsoid model) compared to the proportions for leaves 13 HD for 3306, open circles and 3394, filled circles.

Table 3 – Parameter values for the polynomial curves, Eq. (2), of area in successive sections along leaves of hybrid and planting density combinations shown in Fig. 7

	Parameters		
	k_1	k_2	k_3
3306 LD	71.13 ± 2.58	0.4318 ± 0.1113	-0.0128 ± 0.0011
3306 HD	46.64 ± 1.50	0.6549 ± 0.0577	-0.0102 ± 0.0005
3394 LD	47.89 ± 3.56	0.9595 ± 0.1564	-0.0163 ± 0.0015
3394 HD	31.75 ± 3.47	1.0961 ± 0.1393	-0.0140 ± 0.0012

All parameter estimates are significant at $p < 0.001$.

distributions of three prolate ellipsoids with χ values 0.82 (more horizontal), 0.54 and 0.25 (more vertical) and compare them visually to the angular distributions of leaf 13 at HD for 3306 and 3394. Note that following other researchers who use ellipsoid distributions all leaf area is categorized as lying between 0° and 90° from the horizontal so that an inclination of 5° from the horizontal in Fig. 8 is equivalent to declinations from the stem of both 85° and 95° and areas from both declination angles are summed to calculate contribution to the 5° inclination. For 3306 the contributions to the angular distribution between 0° and 45° inclination from the horizontal are closely approximated by a prolate ellipsoid of $\chi = 0.82$ and similarly, $\chi = 0.54$ for 3394 (Fig. 8). However, in both cases the prolate ellipsoid distribution substantially underestimates the values in the region of the data maxima, i.e., between 50° and 70° inclination, and overestimates for leaf inclination >70°. Both hybrids have their maxima for inclination angles between 50° and 70°. The difference between them is that the proportion of leaf area in this interval relative to that <50° is greater for 3394. For leaves of this type the prolate ellipsoid model is intrinsically biased. Its one parameter, χ , which controls the elongation of the spheroid, can only increase the relative proportion of upright to more horizontal surface by also increasing the angle of inclination at which that maximum occurs and in the case of 3394 moving that maximum to an angle of inclination at which no leaf area occurs.

An appropriate descriptive model is the Richards function (Richards, 1959) fitted to the cumulative distribution of proportional leaf area in relation to leaf inclination angle (Fig. 10):

$$y = \frac{1}{(1 + k_3 e^{-k_2(x-k_1)})^{1/k_3}} \tag{3}$$

where the maximum value is 1, k_1 locates the point of maximum steepness, k_2 is the steepness of the curve and k_3 is the shape parameter. The equation fits to a range of different frequency distributions with 3394 HD having the most upright and 3306 LD having the least upright leaves. Estimates of parameter values with 95% confidence interval values for the graphs in Fig. 10 are given in Table 4.

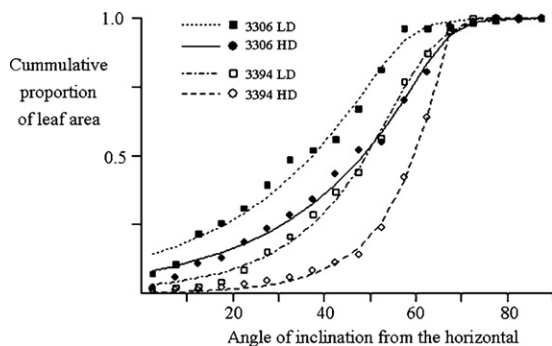


Fig. 10 – The cumulative proportion of leaf area by angle of inclination from the horizontal for six replicates pooled for leaf 13 LD and HD for hybrids 3394 and 3306 (Experiment 2). Lines represent the fitted Richards function for each hybrid. See text for parameter values.

Table 4 – Parameter values and 95% confidence intervals for the Richards functions fitted to the cumulative distribution of proportion of foliage area by inclination angle (Fig. 9)

	Parameters		
	k_1	k_2	k_3
3306 LD	54.43 ± 2.48	0.2241 ± 0.079	3.672 ± 1.656
3306 HD	59.57 ± 4.65	0.3194 ± 0.255	8.097 ± 7.017
3394 LD	48.99 ± 6.39	0.2530 ± 0.221	7.012 ± 6.929
3394 HD	65.27 ± 1.40	1.125 ± 0.765	12.82 ± 8.98

The Richards function provides the flexibility of defining an angle to cumulative area relationship when the maximum occurs at less than 90° and there are different rates of increase at lower angles of inclination for different hybrids. The significance of there being little leaf area at inclinations >75°, and the importance of estimating this correctly is discussed below.

In the torque around the point of attachment of the leaf to the stem (Table 2) there are significant differences ($p < 0.001$) between hybrids, 3306 > 3394, and planting density, LD > HD, but no significant differences between leaf positions and no significant interaction effects. The greater erectness of leaves for 3394 compared to 3306 and for HD compared to LD might be due, in part at least, to lower leaf weight but particularly the distribution of weight from the plant stem that translates into significantly lower torque for 3394.

3.2.2. Midrib anatomy

For leaves 12 and 14 cross-sectional area of the midrib was measured at 5 and 45 cm from the point of attachment of the leaf to the stem (Table 5). Midrib area is significantly greater for LD compared to HD plants except for leaf 12 at 45 cm; however, there are no significant differences between hybrids and no significant interaction effects. The second moment of area (Niklas, 1992, Chapter 3) can be used, along with elastic modulus, to estimate resistance to deformation. While there are differences between planting density in this second moment for the cross-sectional area of midrib, which follow the same pattern as differences in cross-sectional area itself, there are no significant differences between hybrids.

Along the abaxial side of the midrib is a layer of sclerenchyma and veins (Fig. 11). The veins are of three sizes, large, medium and small, as measured by the right angle

Table 5 – Results of ANOVA for leaf midrib cross-sectional area, mm², at 5 and 45 cm from the junction with the stem for leaf 12 and leaf 14.

	5 cm		45 cm	
	Leaf 12	Leaf 14	Leaf 12	Leaf 14
3306 LD	38.3a	33.9a	7.8a	6.1a
3394 LD	37.4a	38.9a	10.8a	7.7a
3306 HD	22.4b	16.9b	6.9a	4.2b
3394 HD	29.6b	20.6b	7.7a	4.9b

Within each column mean values with the same letter do not differ significantly between treatments at $p < 0.01$ on the basis of Tukey's multiple comparison test. There were no differences significant at $p < 0.05$ that were not significant at $p < 0.01$.

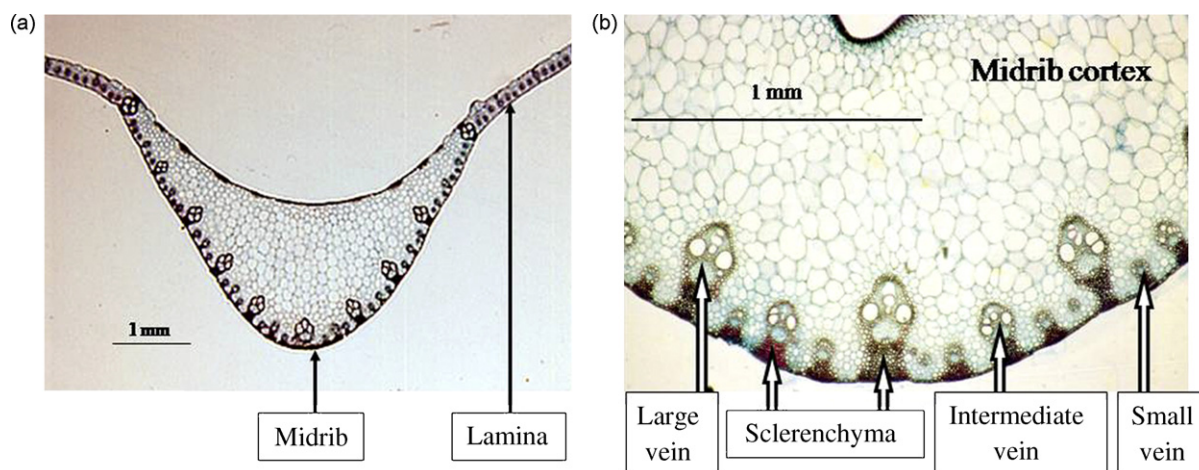


Fig. 11 – (a) Cross-section of a maize leaf midrib at 45 cm from the leaf attachment to the stalk (HD 3394). The area calculated as midrib is delimited by large vascular bundles between the midrib and lamina. (b) Illustration of large, intermediate and small vein sizes and their spatial arrangement along the abaxial side of the midrib.

distance from the epidermis to the inside apex of the vein and the sclerenchyma is continuous between the epidermis and the base of the vein. There are significantly ($p < 0.01$) more total veins mm^{-1} at HD compared to LD for both leaves and at both positions on the leaf (Table 6) and for all leaves, planting densities and hybrids there were more veins mm^{-1} at 45 cm compared to 5 cm. For vascular bundles of all three sizes ANOVA shows the distance from the abaxial surface of the leaf to the interior apex of xylem bundles is larger for LD compared to HD (data not shown), but no consistent differences between hybrids across leaves and distance along the leaf: e.g., for small veins of leaf 12 at 5 cm the distances in mm are 3306 LD 0.196, 3394 LD 0.211, 3306 HD 0.147, 3394 HD 0.156 with a least significant difference of 0.058 ($p < 0.05$).

3.3. Experiment 3

In the second greenhouse experiment hybrid 3394 again had a significantly greater mean H and $H:T$ (leaves 5, 6 and 7) than hybrids 307 and 3306 which do not differ (Table 7). There are marked differences in auricle shape among hybrids (Table 7, Fig. 12). Hybrid 3306 has significantly larger auricle size ($p < 0.001$) than hybrids 307 and 3394, which do not differ, while hybrid 3394 has a significantly ($p < 0.001$) smaller auricle

angle, as defined in Fig. 12, than the other hybrids which do not differ between themselves. There are significant differences between leaves ($p < 0.01$) in auricle base length, with leaf 5 < leaf 6 < leaf 7.

4. Discussion

4.1. How can leaf curvature be defined and does it vary among selected hybrids?

The differences among hybrids in leaf curvature are consistent in general features with those for juvenile leaves of these same lines described previously (Fellner et al., 2003), and with the trends reported for maize over decades (Duvick et al., 2004). In all cases the modern hybrid 3394 has more erect foliage. The empirical measures of H and $T:H$ both showed differences in erectness between hybrids in greenhouse experiments and at two planting densities in the field experiment. Interestingly leaves at all leaf positions, 7 through 14, are more upright for 3394—we did not find leaves at or above position 12 more upright and those at positions 11 and below more horizontal (see also Fakorede and Mock, 1978) as has been suggested for maize ideotypes.

Table 6 – Results of ANOVA of total veins mm^{-1} along the abaxial side of the midrib for leaves at 5 and 45 cm from the point of connection to the stem for leaves 12 and 14

	5 cm		45 cm	
	Leaf 12	Leaf 14	Leaf 12	Leaf 14
3306 LD	3.29ac	3.23a	4.19a	4.14a
3394 LD	2.89a	2.45a	5.39ab	3.73a
3306 HD	3.92b	4.53b	5.24ab	5.10b
3394 HD	3.51bc	4.01b	6.76a	5.35b

Within each column mean values with the same letter do not differ significantly between treatments at $p = 0.05$ on the basis of Tukey's multiple comparison test.

Table 7 – Differences between hybrids in mean characters of leaves 5, 6 and 7, H and $T:H$ as defined in Fig. 1

Hybrid	H (cm)	$T:H$	Auricle base length (mm)	Auricle angle
307	22.88a	-0.687a	16.71a	44.70a
3306	22.06a	-0.398a	19.60b	44.47a
3394	40.87b	0.543b	17.56a	34.13b

The base length of the auricle, i.e., the length along the top of the leaf sheath, and auricle angle, i.e., between leaf sheath and leaf blade, are illustrated in Fig. 7. Within each column mean values with the same letter do not differ significantly between treatments at $p = 0.05$ on the basis of Tukey's multiple comparison test.

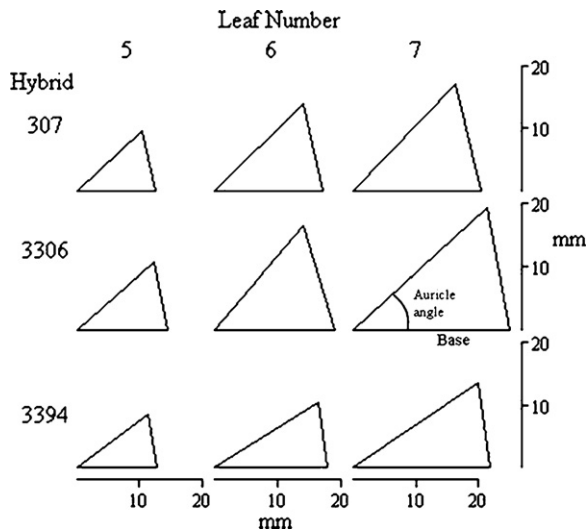


Fig. 12 – Diagrams of auricle dimensions for leaves 5, 6 and 7 of hybrids 307, 3306 and 3394 constructed from mean dimensions. The auricle base, represented as the horizontal line, is the dimension along the distal end of the leaf sheath and the auricle angle refers to the angle between the base and the leaf blade. **Table 6** presents ANOVA results for auricle angle and base length between hybrids.

A significant finding is that in addition to differences in curvature leaves of 3394 are shorter with smaller area. Increased planting density in the field experiment increased leaf length, decreased leaf area, decreased leaf weight and increased both H and $T:H$ for both 3306 and 3394. However, as shown by significant interaction terms in ANOVA for hybrid \times planting density, at increased planting density 3394 decreased leaf area more ($p < 0.05$), increased H more ($p < 0.01$), and increased $T:H$ more ($p < 0.001$) than 3306. This suggests that as well as there being non-plastic differences between the hybrids in leaf posture the plastic response to density is greater for 3394 and the differences in leaf posture are paralleled by differences in leaf area and weight.

4.2. How might variation in leaf curvature be produced?

Although leaves of 3394 are significantly more erect than those of 3306, we found no consistent differences among hybrids in the components of midrib morphology or anatomy that might explain this. While there were significant differences in vascular bundle dimensions of the midrib between hybrids these were not consistent, i.e., hybrid 3394 cannot be considered simply to have a stronger midrib from the characters of midrib dimensions and vascular bundles we measured. At 5-cm distance from the stem 3394 has taller large, intermediate and small veins—but with consistent significance only for leaf 14 but it also has significantly fewer veins mm^{-1} (Table 5). At 45 cm along the leaf the reverse is found. 3394 has significantly more veins mm^{-1} for leaf 12 but where significant differences between hybrids occur in vein size then $3306 > 3394$.

A component of the explanation for differences in curvature may be differences in leaf length, area, weight and auricle structure. Leaves of hybrid 3394 have a lower torque. Our calculations of torque are made using dry weights and further investigations are needed using *in situ* wet weight calculations and how torque may change as leaves grow. If differences in torque are sufficient to cause differences in irreversible bending during leaf development, perhaps through an effect on auricle development, then there may be no requirement to seek explanations of leaf erectness in terms of midrib dimensions and anatomy. The differences in auricle angle are likely to have been established during the early phase of sheath growth (Osmont et al., 2003) and, combined with differences in leaf length and width, may establish different patterns for the progression of irreversible bending during leaf development.

From the results of manipulative experiments of light quality and application of auxin Fellner et al. (2003, 2006) propose the underlying physiological difference between 3394 and the two older hybrids is in responsiveness to auxin. Leaf declination from the vertical was reduced by application of the auxin transport inhibitor NPA and this effect was greater in the older hybrids, 307 and 3306, than in the modern hybrid 3394. The diameter of xylem vessels was greater in 3394, and was unaffected by NPA, whereas in the older hybrids, the normally smaller vessels were enlarged by treatment with NPA. This explanation operates at the cell and leaf level (i.e., systemically). If confirmed in other hybrids with more upright leaves and, given that all leaves in 3394 are more upright than those of older hybrids, it would suggest that what has been implicitly selected is reduced auxin sensitivity. Consequently, selection for upright leaves in the upper part of the plant, and, at the same time more horizontal leaves in the lower part, as suggested for some maize ideotypes, would likely be unsuccessful.

4.3. How can the relationship between leaf area and its angle of inclination be represented?

Curvature of the complete leaf could not be described with a single mathematical function, or even two functions of conic sections as suggested by other researchers (Prévoit et al., 1991; Stewart and Dwyer, 1993). Curvature between the point of attachment to the stem and leaf maximum height could be approximated by an exponential function but the leaf surface distal to that point was irregular.

We find a prolate ellipsoid model does not fit the leaf area distribution by angle of individual leaves. For example, with $\chi = 0.82$, this model considerably underestimated the maximum area for leaf 13 of 3306; with $\chi = 0.54$ this model underestimated the maxima, of leaf 13 of 3394 and had its maximum 10° higher (Fig. 9) although in both cases the model distributions tracked the distributions between 0° and 45° . Antunes et al. (2001) suggest a spherical distribution of leaf area by angle for whole plants. However, for one of the two sets of data they illustrate, there is a marked maximum in the data at 65° , exceeding that for the spherical distribution and data values for all angles between 15° and 55° are less.

We found that empirical data of leaf area by inclination angle could be fit by Richards functions (Fig. 10) and for leaf 13 of field-

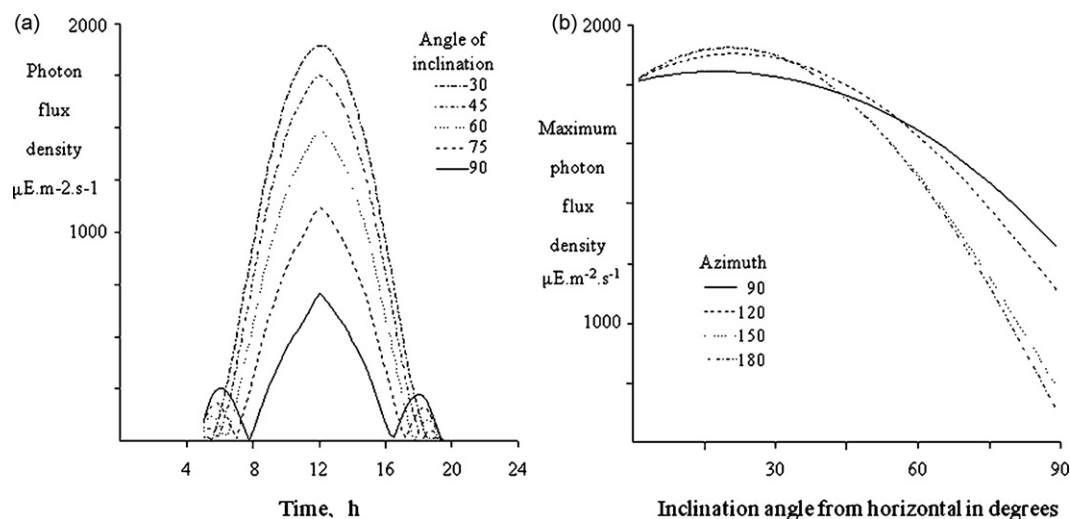


Fig. 13 – (a) Daily course of calculated photon flux density received by direct radiation for summer day, 14 July 2002, at Johnson, Iowa, for planes at different angles of inclination and with azimuth towards maximum sun elevation. (b) Maximum calculated photon flux density received during a mid-summer day, Johnson, Iowa, for inclined planes at different azimuths.

grown maize (Experiment 2) both 3306 and 3394 show a maximum inclination angle of ~65°. Correct estimation of the maximum area of inclination at which foliage occurs is important. Calculation of the course of daily photon flux density, for a summer day when all leaves are fully formed, and for surfaces oriented towards the azimuth of solar noon show a marked decline for inclinations >60° (Fig. 13a). Calculation of interception on a surface is dependent on angle, and azimuth between beam and surface as given by Idso and de Wit (1970), their Appendix A, and the intensity of the beam as calculated from Gates (1980). We did not include an estimate for diffuse light in this calculation given the difficulty in calculating it for different times of day so that our estimates of hours of photon flux density will be an under-, rather than over-estimate. During the course of a mid-July day surfaces with inclinations greater than 60° receive markedly lower photon flux densities. When surface azimuth is taken into account and maximum photon flux density over a day is calculated a marked decline can also be seen for inclination angles >60° (Fig. 13b). This non-linear decline for surfaces >60° is particularly interesting given that the maximum inclination angle found, for both hybrids, is 60°, and that a greater proportion of leaf area is closer to this angle for hybrid 3394 than 3306.

Acknowledgements

Seeds for all experiments were provided by Pioneer Hi Bred Intl. (Des Moines, IA, USA). We wish to thank Pioneer Hi Bred Intl. for funding and Chris Zinzelmair for his advice and help.

REFERENCES

Antunes, M.A.H., Walter-Shea, E.A., Mesarch, M.A., 2001. Test of an extended mathematical approach to calculate maize leaf

area index and leaf angle distribution. *Agric. Forest Meteorol.* 108, 45–53.

Campbell, G.S., 1986. Extinction coefficients for radiation in plant canopies calculated using an ellipsoidal inclination angle distribution. *Agric. Forest Meteorol.* 36, 317–321.

Campbell, G.S., 1999. Derivation of an angle density function for canopies with ellipsoidal leaf angle distributions. *Agric. Forest Meteorol.* 49, 173–176.

Chambers, J.M., Hastie, T.J. (Eds.), 1992. *Statistical Models in S*. Wadsworth & Brooks/Cole, Pacific Grove, CA.

Comprehensive R Archive Network, 2008. *The R Project for Statistical Computing*. <http://www.r-project.org/>.

Duncan, W.G., 1971. Leaf angle, leaf area and canopy photosynthesis. *Crop Sci.* 11, 482–485.

Duvick, D.N., Smith, J.S.C., Cooper, M., 2004. Long-term selection in a commercial hybrid maize breeding program. In: Janick, J. (Ed.), *Plant Breeding Reviews 24 (Part 2)*. Long Term Selection: Crops, Animals, and Bacteria. John Wiley & Sons, New York, pp. 109–151.

España, M.L., Baret, F., Aries, F., Chelle, M., Andrieu, B., Prévot, L., 1999. Modeling maize canopy 3D architecture Application to reflectance simulation. *Ecol. Model.* 122, 25–43.

Fakorede, M.A.B., Mock, J.J., 1978. Changes in morphological and physiological traits associated with recurrent selection for grain yield in maize. *Euphytica* 27, 397–409.

Fellner, M., Horton, L.A., Cocke, A.E., Stephens, N.R., Ford, E.D., Van Volkenburgh, E., 2003. Light interacts with auxin during leaf elongation and leaf angle development in young corn seedlings. *Planta* 216, 366–376.

Fellner, M., Ford, E.D., Van Volkenburgh, E., 2006. Development of erect leaves in a modern maize hybrid is associated with reduced responsiveness to auxin and light of young seedlings in vitro. *Plant Signaling Behavior* 1, 204–211.

Freeling, M., 1992. A conceptual framework for maize leaf development. *Dev. Biol.* 153, 44–58.

Gates, D.M., 1980. *Biophysical Ecology*. Springer-Verlag, New York.

Hawkins, A.F., 1982. Light interception, photosynthesis and crop productivity. *Outlook Agric.* 11, 104–113.

- Hay, J.O., Moulia, B., Lane, B., Freeling, M., Silk, W.K., 2000. Biomechanical analysis of the Rolled (*Rld*) leaf phenotype of maize. *Am. J. Bot.* 87, 625–633.
- Idso, S.B., de Wit, C.T., 1970. Light relations in plant canopies. *Appl. Optics* 9, 177–184.
- Lizaso, J.I., Batchelor, W.D., Boote, K.J., Westgate, M.E., 2005. Development of a leaf-level canopy assimilation model for CERES-Maize. *Agron. J.* 97, 722–733.
- Long, S.P., Zhu, X.G., Naidu, S.L., Ort, D.R., 2006. Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* 29, 315–330.
- Mock, J.J., Pearce, R.B., 1975. An ideotype of maize. *Euphytica* 24, 613–623.
- Monteith, J.L., 1969. Light interception and radiative exchange in crop stands. In: Eastin, J.D., Haskins, F.A., Sullivan, C.Y., van Bavel, C.H.M. (Eds.), *Physiological Aspects of Crop Yield*. American Society of Agronomy and Crop Science, Madison, Wisconsin, pp. 89–115.
- Monteith, J.L., 1973. *Principles of Environmental Physics*. Edward Arnold, London.
- Moulia, B., Fournier, M., Guitard, D., 1994. Mechanics and form of the maize leaf: *in vivo* qualification of flexural behaviour. *J. Mater. Sci.* 29, 2359–2366.
- Moulia, B., Fournier, M., 1997. Mechanics of the maize leaf: a composite beam model of the midrib. *J. Mater. Sci.* 32, 2771–2780.
- Niklas, K.J., 1992. *Plant Biomechanics*. University of Chicago Press, Chicago.
- Osmont, K.S., Jesaitis, L.A., Freeling, M., 2003. The *extended auricle1 (eta1)* gene is essential for the genetic network controlling post initiation maize leaf development. *Genetics* 165, 1507–1519.
- Pendleton, J.W., Smith, G.E., Winter, S.R., Johnston, T.J., 1968. Field investigations of the relationship of leaf angle in corn (*Zea mays* L.) to grain yield and apparent photosynthesis. *Agron. J.* 60, 422–424.
- Pepper, G.E., Pearce, R.B., Mock, J.J., 1977. Leaf orientation and yield of maize. *Crop Sci.* 17, 883–886.
- Prévot, L., Arie, F., Monestiez, P., 1991. Modélisation de la structure géométrique di maïs. *Agronomie* 11, 491–503.
- Richards, F.J., 1959. A flexible growth function for empirical use. *J. Exp. Bot.* 10, 290–300.
- Ritchie, S.W., Hanaway, J.J., Benson, G.O., 1993. How a corn plant develops. Special Report 48. Iowa State University, Ames, Iowa, USA.
- Sass, J.E., 1958. *Botanical Microtechnique*, 3rd ed. Iowa State College Press, Ames, Iowa.
- Sharman, B.C., 1942. Developmental anatomy of the shoot of *Zea mays* L. *Ann. Bot.* 6, 245–282.
- Silk, W.K., Erickson, R.O., 1978. Kinematics of hypocotyls curvature. *Am. J. Bot.* 65, 310–319.
- Sinclair, T.R., Muchow, R.C., 1999. Radiation use efficiency. *Adv. Agron.* 65, 215–265.
- Smith, J.S.C., Duvick, D.N., Smith, O.S., Cooper, M., Feng, L., 2004. Changes in pedigree backgrounds of Pioneer brand maize hybrids widely grown from 1930 to 1990. *Crop Sci.* 44, 1935–1946.
- Stewart, D.W., Dwyer, L.M., 1993. Mathematical characterization of maize canopies. *Agric. Forest Meteorol.* 66, 247–265.
- Stewart, D.W., Costa, C., Dwyer, L.M., Smith, D.L., Hamilton, R.I., Ma, B.L., 2003. Canopy structure, light interception, and photosynthesis in maize. *Agron. J.* 95, 1465–1474.
- Winter, S.R., Ohlrogge, A.J., 1973. Leaf angle, leaf area, and corn (*Zea mays* L.) yield. *Agron. J.* 65, 395–397.
- Zar, J.H., 1999. *Biostatistical Analysis*, 4th ed. Prentice Hall, Upper Saddle River, NJ.