Co-ordination between Leaf Initiation and Leaf Appearance in Field-grown Maize (Zea mays): Genotypic Differences in Response of Rates to Temperature

J. M. PADILLA1,* and M. E. OTEGUI1,2

1Facultad de Agronomía, UBA, Av. San Martín 4453 (C1417DSE), Buenos Aires, Argentina and
2CONICET, Av. Rivadavia 1917 (C1033AA), Buenos Aires, Argentina

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• Background and Aims In maize (Zea mays), early flowering date, which is a valuable trait for several cropping systems, is associated with the number of leaves per plant and the leaf appearance rate. Final leaf number depends upon the rate and duration of leaf initiation. The aims of this study were to analyse the genotypic variation in the response to temperature of leaf appearance rate and leaf initiation rate, and to investigate the co-ordination between these processes under field conditions.

• Methods Sixteen hybrids of different origins were grown under six contrasting environmental conditions. The number of appeared leaves was measured twice a week to estimate leaf appearance rate (leaves d−1). Plants were dissected at four sampling dates to determine the number of initiated leaves and estimate leaf initiation rate (leaves d−1). A co-ordination model was fitted between the number of initiated leaves and the number of appeared leaves. This model was validated using two independent data sets.

• Key Results Significant (P < 0.05) differences were found among hybrids in the response to temperature of leaf initiation rate (plastochron) and leaf appearance rate (phylochron). Plastochron ranged between 24.3 and 36.4 degree days (°Cd), with a base temperature (Tb) between 4.0 and 8.2 °C. Phylochron ranged between 48.6 and 65.5 °Cd, with a Tb between 2.9 and 5.0 °C. A single co-ordination model was fitted between the two processes for all hybrids and environments (r² = 0.96, P < 0.0001), and was successfully validated (coefficient of variation < 9 %).

• Conclusions This work has established the existence of genotypic variability in leaf initiation rate and leaf appearance rate in response to temperature, which is a promising result for maize breeding; and the interdependence between these processes from seedling emergence up to floral initiation.

Key words: Zea mays, maize, co-ordination, leaf initiation, leaf appearance, plastochron, phyllochron, Tb, genotypic variability, temperature, modelling.

INTRODUCTION

In maize (Zea mays), early silking (i.e. female flowering) is a valuable trait for many cropping systems. For example, in high-latitude environments (i.e. >45°) the potential length of the growing cycle is strongly dependent upon sowing date, which is generally late in spring when soil temperatures do not present a risk to successful seedling establishment (Carr and Hough, 1978). This restricts yield potential, especially because silking usually takes place after the highest input of solar radiation at the summer solstice, and both the supply of solar radiation and temperature decline sharply during grain filling (Otegui and Bonhomme, 1998). Early silking is also important in some rain-fed mid-latitude environments, to avoid the risk of water stress during the critical period for grain set around flowering (Hall et al., 1981).

The time elapsing between sowing and silking is associated with the number of leaves per plant and the rate of leaf appearance (Tollenaar et al., 1979). Reductions in leaf number that advance silking date imply a reduction in plant size (Dijak et al., 1999), and the need to increase stand density to achieve maximum light interception at the start of the critical period for grain set (Otegui and Bonhomme, 1998); this, in turn, would reduce the amount of light intercepted per plant, with potential negative effects on final grain number per unit area (Loomis and Connor, 1992). The rate of leaf appearance is determined primarily by temperature: developmental rates are linearly related to temperature in the range between a base temperature (Tb), at which no significant development can be detected, and an optimum temperature (To), at which the developmental rate is maximal (Ritchie and NeSmith, 1991). Within this range, the thermal time interval between the appearance of successive leaf tips is defined as the phyllochron (McMaster and Wilhelm, 1995). Therefore, one strategy for improving the adjustment of the crop to the environment could be to search for genetic variability in phyllochron and Tb.

Published values of maize phyllochron generally fall between 37 and 42 degree days (°Cd), with a Tb of 8 °C (Hesketh and Warrington, 1989; Otegui and Melón, 1997), and Birch et al. (1998) obtained values of 35 and 50 °Cd for different maize hybrids and populations, although the differences were mainly among environments. Verheul et al. (1996) found slightly greater variation in phyllochron values (between 38 and 52 °Cd) among inbred lines than those quoted for hybrids, but meaningful comparisons are difficult because they used a Tb of 6 °C. Giauffret et al. (1995) found that not only the phyllochron but also the Tb could vary...
among inbred lines, with values ranging between 33 and 62 °Cd for the former and between 7 and 12.5 °C for the latter. All of these values should, however, be treated with caution as they are based on air temperature, whereas the apical meristem is below the soil surface during early growth (Ritchie and NeSmith, 1991; Vinocur and Ritchie, 2001).

Final leaf number depends upon the rate and duration of leaf initiation. The rate is usually calculated from the quotient of the number of leaves produced from sowing until tassel initiation and the time elapsed between these events, taking into account the number of leaves already present in the embryo, usually taken to be five (Hunter et al., 1977) or six (Aitken, 1980). Warrington and Kanemasu (1983) calculated the rate of leaf initiation from frequent observations of leaf primordia production by the apex, finding no differences between the two hybrids examined, and determining a plastochron (interval of thermal time between the initiation of successive primordia) of 20.6 °Cd and a \( T_b \) of 7.3 °C (Hesketh and Warrington, 1989). Evidently, more information is needed on plastochron values of hybrids currently bred for different environments (i.e. with varying final leaf number), as well as for the number of leaf primordia present in the embryo and the actual \( T_b \) above which significant development can be detected.

Early research on genotypic differences in crop life cycle among maize cultivars established a strong relationship between the durations (in thermal time) of two developmental phases: sowing to tassel initiation and sowing to tassel emergence (Kiniry et al., 1983). The relationship between these phases was observed for a wide range of cultivars and photoperiods, which suggested an association between the rates of leaf initiation and of leaf appearance. Tollenaar and Hunter (1983) found that the number of appeared leaves by tassel initiation usually represented 50% of the final leaf number under a range of photoperiods and temperature regimes. Little is known, however, about genotypic differences in the relationship between leaf initiation and leaf appearance from seedling emergence to tassel initiation.

The aims of this study were (a) to analyse the genotypic variation in the response to temperature of the rates of leaf initiation and appearance in a set of commercial hybrids bred for contrasting cropping environments, and (b) to investigate the possible co-ordination of these processes under field conditions. Sixteen hybrids of different origins (USA, Europe and Argentina) were included in the analysis, and were sown at five contrasting sowing dates to obtain a wide range of temperatures early in the crop life cycle (i.e. up to tassel initiation).

**MATERIALS AND METHODS**

**Crop husbandry and experimental design**

Five experiments were conducted between 2002 and 2004 at the experimental farm of the University of Buenos Aires (34°35’S, 58°29’W), Argentina, on a silty clay loam soil (Vertic Argiudoll). Sowing took place on 23 August 2002 (expt 1), 10 October 2002 (expt 2), 25 March 2003 (expt 3), 26 August 2003 (expt 4) and 2 January 2004 (expt 5).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Origin</th>
<th>Seed source</th>
<th>Final leaf no.*</th>
<th>TT emergence to physiological maturity (°Cd)†,‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>33G26</td>
<td>USA</td>
<td>Pioneer</td>
<td>19-0</td>
<td>1749</td>
</tr>
<tr>
<td>34B15</td>
<td>USA</td>
<td>Pioneer</td>
<td>20-0</td>
<td>1709</td>
</tr>
<tr>
<td>34K77</td>
<td>USA</td>
<td>Pioneer</td>
<td>19-5</td>
<td>1709</td>
</tr>
<tr>
<td>34N16</td>
<td>USA</td>
<td>Pioneer</td>
<td>18-7</td>
<td>1762</td>
</tr>
<tr>
<td>36G12</td>
<td>USA</td>
<td>Pioneer</td>
<td>17-1</td>
<td>1679</td>
</tr>
<tr>
<td>39A26</td>
<td>USA</td>
<td>Pioneer</td>
<td>16-7</td>
<td>1448</td>
</tr>
<tr>
<td>39R52</td>
<td>USA</td>
<td>Pioneer</td>
<td>16-5</td>
<td>1491</td>
</tr>
<tr>
<td>ANJOU 258</td>
<td>Europe</td>
<td>Monsanto</td>
<td>15-1</td>
<td>1491</td>
</tr>
<tr>
<td>MONUMENTAL</td>
<td>Europe</td>
<td>Monsanto</td>
<td>15-9</td>
<td>1529</td>
</tr>
<tr>
<td>DK312</td>
<td>Europe</td>
<td>Monsanto</td>
<td>18-2</td>
<td>1575</td>
</tr>
<tr>
<td>DK315</td>
<td>Europe</td>
<td>Monsanto</td>
<td>17-6</td>
<td>1514</td>
</tr>
<tr>
<td>DK615</td>
<td>Argentina</td>
<td>Monsanto</td>
<td>19-9</td>
<td>1802</td>
</tr>
<tr>
<td>DK664MG</td>
<td>Argentina</td>
<td>Monsanto</td>
<td>19-6</td>
<td>1804</td>
</tr>
<tr>
<td>DK682</td>
<td>Argentina</td>
<td>Monsanto</td>
<td>20-8</td>
<td>1815</td>
</tr>
<tr>
<td>DK752</td>
<td>Argentina</td>
<td>Monsanto</td>
<td>21-5</td>
<td>1866</td>
</tr>
<tr>
<td>DK834</td>
<td>Argentina</td>
<td>Monsanto</td>
<td>24-9</td>
<td>2004</td>
</tr>
<tr>
<td>B7³</td>
<td>ISU³</td>
<td>USA, FAUBA³</td>
<td>21-4</td>
<td>1873</td>
</tr>
<tr>
<td>Mo17⁴</td>
<td>MU⁴, USA, FAUBA⁴</td>
<td></td>
<td>19-0</td>
<td>1804</td>
</tr>
<tr>
<td>B7³ × Mo17⁴</td>
<td>USA, FAUBA⁴</td>
<td></td>
<td>19-6</td>
<td>1781</td>
</tr>
</tbody>
</table>

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<tr>
<th>Data are means of two sowing dates (expts 4 and 5) with a day length of 16 h between seedling emergence and tassel initiation.</th>
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</thead>
<tbody>
<tr>
<td>† Thermal time (TT) in degree days (°Cd) was estimated as: TT = ( \sum_{i=1}^{n} (T - T_b) ), where ( T ) is mean daily air temperature between seedling emergence and physiological maturity, the base temperature ( T_b ) was assumed to be 8°C, and ( n ) is the number of days elapsed.</td>
</tr>
<tr>
<td>‡ Genotype controls.</td>
</tr>
</tbody>
</table>

Sixteen hybrids of *Zea mays* L. of varying maturity type, origin and seed source were included in all experiments (Table 1). Inbred lines B73 and Mo17, and their hybrid B73 × Mo17 were included only in expts 4 and 5. The crop stands were fertilized with 16.2 kg N ha⁻¹ and 17.9 kg P ha⁻¹ at sowing. Four applications, each of 50 kg N ha⁻¹, were also added at VT₆ (six visible leaf tips), VT₁₀, VT₁₄ and VT₁₇ in expts 4 and 5.

Experiment 1 was a factorial combination of two planting systems (seeds pre-germinated in a controlled environment, or not pre-germinated) and the 16 hybrids. Treatments were distributed in the field in a split-plot design with three replicates, with planting systems as main plots and genotypes as subplots. Pre-germination was performed in plastic boxes in irrigated sand; seeds attached to an adhesive paper ribbon were exposed to 21.6 ± 0.6 °C for 48 h, plus 72 h at 13.4 ± 0.1 °C. This treatment was included to ensure a short interval between planting and seedling emergence, and the exposure of seedlings to the low temperatures of late winter, in August. Each sub-plot had one (pre-germinated seeds) and two (non-pre-germinated seeds) rows 2 m long, 0.175 m apart, and plants spaced at 0.2 m within the row (i.e. initial stand density of 28 plants m⁻²). In non-pre-germinated subplots, seeds were distributed in the soil by two means: (1) attached to an adhesive paper ribbon at a rate of three seeds per site (one row), or (2) direct into the soil at a rate of one seed per site (the other row). The paper-ribbon system was
used to produce an almost perfect seed distribution, indispensable for the accurate determination of the rate of seedling emergence. In all experiments, seeds were sown at a depth of 5 cm.

In the other experiments, the hybrids were distributed in the field in a completely randomized block design with three replicates. Each subplot had three rows. Seeds in the central row were sown by the paper-ribbon system, and those in the other two rows were placed directly into the soil, at the same rate as in exp 1. In all experiments, rows produced by paper-ribbon sowing were thinned to one plant per site immediately after seedling emergence (VT$_3$). In expts 2–5, one direct-sown row had been completely removed by sampling by VT$_6$ and the other by VT$_9$.

The photoperiod was artificially extended to constant values of 15 (expts 1, 2 and 3) or 16 (expts 4 and 5) hours per day throughout the growth of the crops, using four towers, each with one halogen lamp (General Electric, DEQ 500W 118 DISP, Buenos Aires, Argentina). The towers were spaced at 4-m intervals at 1.5 m above the soil level, and at 1.5 m from one side of the experimental area (15 m length × 8 m width). This arrangement gave a mean of 0.18 W m$^{-2}$, with extreme values of 0.25 and 0.16 W m$^{-2}$ (Analytical Spectral Devices, Field Spec Hand Held, Boulder, CO, USA). These values were among the range of 0.05 (less sensitive hybrids) and 0.25 W m$^{-2}$ (more sensitive hybrids) determined as critical by Francis et al. (1970) for maize, and allowed the results to be compared with others in the literature. The mean red/far red ratio (660/730) provided by the lamps was 0.89 with extreme values of 0.92 and 0.85. In confirmation of the appropriateness of this lighting system, the final leaf number of hybrid B73 × Mo17 under a photoperiod of 16 h in expts 4 and 5 (19-6; Table 1) was almost identical to that obtained by Bonhomme et al. (1991) for the same photoperiod (approx. 19-75). Experiments were continued until tassel initiation (expts 1, 2 and 3) or until physiological maturity (expts 4 and 5).

**Measurements**

Seedling emergence (i.e. coleoptile visible above the soil surface) was recorded daily in rows sown with the paper-ribbon sowing system until each subplot reached the VT$_3$ stage. Time of observation was registered, and the time when 50% of the stand reached the stage was calculated by linear interpolation on a 24-h basis (Muthukuda Arachchi et al., 1999).

The number of appeared leaves (tip visible) was registered, from seedling emergence up to tassel initiation, on identified plants in the row under the paper-ribbon sowing system, which were tagged in all subplots at 50% seedling emergence. The number of plants tagged per subplot was four (expt 1 pre-germinated treatment), seven (expt 1 non-pre-germinated treatment) and five (expts 2–5). On each plant, the fifth leaf was tagged to ensure correct measurements of appeared leaves and to avoid errors owing to leaf senescence. Observations were made every 2 or 3 d, depending on air temperature, and data were transformed to a proportional scale to obtain a continuous rather than a discrete variable. By this transformation, a newly appeared leaf tip (leaf n) received a value of

\[ (a) n + 0.25, \quad (b) n + 0.5, \quad (c) n + 0.75 \]

when 1–2 cm of the leaf was visible from a lateral position, >2 cm was visible from a lateral position, and when leaf n + 1 was visible within the whorl but had not yet appeared.

The number of leaf primordia initiated by the apex was measured after destructive plant sampling at the VT$_3$, VT$_6$ and VT$_9$ stages in all experiments. These stages occurred before tassel initiation, except for the hybrid ANJOU 258 (last sampling at VT$_7$ in expts 4 and 5, when the plants had already initiated tassels at VT$_9$). An extra sample was taken after tassel initiation in all cases. Measurements were performed on identified plants, which were tagged in all subplots at 50% seedling emergence. These four plants belonged to the row under the paper-ribbon (expt 1 pre-germinated treatment), to the row under direct sowing (expt 1 non-pre-germinated treatment) or to one of the two rows under direct sowing (expts 2 to 5). Final stand density after all samplings was 9.5 plants m$^{-2}$. Sampled plants were preserved in a standard solution of formaldehyde (100 mL L$^{-1}$), acetic acid (50 mL L$^{-1}$), ethyl alcohol (500 mL L$^{-1}$) in distilled water until they were dissected for apex observation. The number of leaves initiated was counted using a binocular microscope (Leica stereomicroscope, MZ6, Leica Wetzlar, Germany). A leaf primordium was registered as initiated when it represented at least one-third of the apex dome (the ‘primordium mid stage’ of Abbe and Pinney, 1951). The number of leaves present in the embryo was determined in ten pre-germinated (coleoptile length <1 cm) seeds per hybrid.

Soil temperature, at a depth of 5 cm, was recorded hourly in each replicate with sensors (LM35, National Semiconductors, Santa Clara, CA, USA) connected to data-loggers (Temp-Logger, Cavadevices, Buenos Aires, Argentina). Mean daily (average of hourly records) screen air temperature and incident photosynthetically active radiation (PAR) were measured at 2 m above bare soil using sensors (Vaisala thermo-hygrometer, Vaisala, Woburn, MA, USA, and LI-190SB, LI COR, Lincoln, NE, USA) connected to a datalogger (Campbell 21X, Campbell Scientific, Logan, UT, USA) at a meteorological station 300 m away.

**Plastochron and phyllochron estimation**

The leaf initiation rate ($L_{IR}$) was calculated as the slope of the linear relationship between the number of initiated leaves ($N_{IL}$) and the number of days after sowing (Warrington and Kanemasu, 1983). In a similar way, the leaf appearance rate ($L_{AR}$) was obtained as the slope of the linear relationship between the number of appeared leaves ($N_{AL}$) and the number of days after sowing (Thiagarajah and Hunt, 1982). A linear relationship was also fitted between each of the rates of development (in time) and temperature, because the mean temperature in the experiments was within the range of linear response to temperature of both leaf appearance (between 12 and 26°C; Tollenaar et al., 1979) and leaf initiation rates (between 15 and 25°C; Warrington and Kanemasu, 1983). The plastochron...
was estimated as the inverse of the slope of the linear model fitted to the relationship between leaf initiation rate at the dome apex and mean soil temperature at a depth of 5 cm, which is a good substitute for apex temperature until tassel initiation (Vinocour and Ritchie, 2001). The same approach was used for phyllochron estimation, but based on the rate of leaf appearance. The \( T_b \) of each process (i.e. leaf initiation and leaf appearance) was obtained from extrapolation of the corresponding model. \( L_{IR} \) and \( L_{AR} \) were calculated for the period between \( VT_3 \) and \( VT_9 \).

Co-ordination model development

The co-ordination between leaf initiation at the apex and leaf appearance was tested. Data included in the analysis corresponded to (a) plants sampled at \( VT_3 \), \( VT_6 \) and \( VT_9 \) (or \( VT_7.5 \) for hybrid ANJOU 258 in expts 4 and 5), for which the \( N_{IL} \) and the \( N_{AL} \) were recorded; (b) the estimated number of initiated leaves at seedling emergence (\( N_E \)); and (c) the estimated \( N_{AL} \) at tassel initiation.

The \( N_{AL} \) was set to zero at seedling emergence, and the \( N_E \) was computed (eqn 1).

\[
N_E = N_B + \left[ \left( N_3 - N_B \right) \times TT_3^{-1} \right] \times TT_E \tag{1}
\]

where \( N_B \) is the mean number of leaves present in the embryo, \( N_3 \) is the mean number of initiated leaves measured at \( VT_3 \), \( TT_3 \) is thermal time from sowing to \( VT_3 \), and \( TT_E \) is thermal time from sowing to seedling emergence. Thermal time at seedling emergence (\( TT_E \)) and at \( VT_3 \) (\( TT_3 \)) was estimated from eqn (2).

\[
TT(Cd) = \left[ \sum_{i=1}^{n} (T - T_b) \right] \times 0.0416 \times \left( d \times h^{-1} \right) \tag{2}
\]

where \( T \) is hourly recorded soil temperature (\( ^\circ C \)), at a depth of 5 cm, from sowing to the time of seedling emergence (\( TT_E \)) or until \( VT_3 \) (\( TT_3 \)), and \( n \) is the number of hours elapsed. The value of \( T_b \) corresponded to the value estimated for each hybrid from the relationship between \( L_{IR} \) and temperature.

The date of tassel initiation was obtained by extrapolation from the bilinear model fitted to the relationship between the \( N_{IL} \) and thermal time, which included observations taken after tassel initiation. Afterwards, the \( N_{AL} \) at this stage was estimated by linear interpolation.

A bilinear model (eqns 3 and 4) was fitted to the relationship between \( N_{AL} \) and \( N_{IL} \), using the iterative optimization technique of TBL Curve (Jandel Scientific, 1992).

\[
N_{AL} = a + bN_{IL}, \text{ for } N_{IL} \leq c \tag{3}
\]

\[
N_{AL} = a + bN_{IL} + d(N_{IL} - c), \text{ for } N_{IL} > c \tag{4}
\]

where \( a \) is the intercept of the first phase (eqn 3), \( b \) and \( d \) are the slopes of the first and the second (eqn 4) phases, and \( c \) is the breakpoint between these phases.

Co-ordination model validation and statistical analysis

Two independent data sets were used for validation of the co-ordination model. The main objective was to test the ability of the model to predict \( N_{AL} \) between seedling emergence and tassel initiation. One data set was obtained from the inbred lines B73 and Mo17, and their hybrid B73 × Mo17 (five plants of each sampled at \( VT_3 \), \( VT_6 \), \( VT_7.5 \) and \( VT_9 \) in expts 4 and 5). The \( N_{AL} \) was recorded, and then plants were dissected to determine \( N_{IL} \). The second data set was obtained from Tollenaar and Hunter (1983), who recorded the \( N_{AL} \) at tassel initiation and final leaf number of a short-season genotype grown in three experiments under controlled conditions. Their first experiment included seven treatments, involving the transfer of plants between two contrasting temperatures (15 and 35 °C), while the second and third experiments were performed at constant air temperature (30 and 20 °C) and included eight treatments, involving the transfer of plants between two photoperiods (10 and 20h).

The accuracy of the model predictions was tested by mean squared deviation (\( M_{SD} \)) between predicted and observed number of appeared leaves (Kobayashi and Salam, 2000). The \( M_{SD} \) was partitioned into the bias between predicted and observed number of appeared leaves (i.e. squared bias, \( S_B \)); the ability of the model to simulate the magnitude of the fluctuation between predicted and observed number of appeared leaves (i.e. squared difference between standard deviations, \( S_{DSD} \)); and the pattern of the fluctuation among the \( n \) measurements (i.e. lack of correlation weighted by the standard deviations, \( L_{CS} \)). These components are described in eqns (5)–(10).

\[
M_{SD} = \frac{1}{n} \sum (x_i - y_i)^2 = S_B + S_{DSD} + L_{CS} \tag{5}
\]

\[
S_B = (\bar{x} - \bar{y})^2 \tag{6}
\]

\[
S_{DSD} = (S_{DS} - S_{Dm})^2 \tag{7}
\]

\[
L_{CS} = 2S_{DS}S_{Dm}(1 - r) \tag{8}
\]

\[
S_{DS} = \sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2} \tag{9}
\]

\[
S_{Dm} = \sqrt{\sum_{i=1}^{n} (y_i - \bar{y})^2} \tag{10}
\]

where \( y \) represents the observed number of appeared leaves, \( x \) is the predicted number of appeared leaves, \( r \) is the correlation coefficient between them, \( S_{DS} \) is the standard deviation of the predicted number of appeared leaves and \( S_{Dm} \) is the standard deviation of the observed number of appeared leaves.

Differences among hybrids in the response to temperature of the rates of leaf initiation and leaf appearance were compared by means of paired \( F \) tests (\( F < 0.05 \); Steel and Torrie, 1992). Genotypic variability in the model parameters (i.e. phyllochron, plastochron and \( T_b \)) was not tested in this study owing to the difference between the low-end temperature explored in the experiments (expt 1 pre-germinated...
Two aspects of the growing conditions deserved attention: the similar temperature regime of expts 2, 3 and 4 (Fig. 1A), whereas the input of PAR varied. This was owing to the contrasting irradiance (a) during spring between years 2002 (expt 2) and 2003 (expt 4), and (b) between spring (expts 2 and 4) and autumn (expt 3) sowings. Median PAR values were relatively high for expt 2 (10.6 MJ m\(^{-2}\) d\(^{-1}\)), intermediate for expt 4 (7.3 MJ m\(^{-2}\) d\(^{-1}\)) and low for expt 3 (4.3 MJ m\(^{-2}\) d\(^{-1}\)). By contrast, expts 2 and 5 had similar inputs of PAR (Fig. 1B), but median temperature in expt 2 was 7.4°C lower than in expt 5.

**Plastochron and phyllochron**

For each treatment, the rates of leaf initiation and of leaf appearance were constant during the period of study, and the linear relationships with days after sowing of both the number of initiated leaves and the number of appeared leaves were highly significant \(r^2 > 0.98\) in most experiments. The only exception was for the pre-germinated seeds of expt 1, for which a bilinear model gave a better fit \(r^2 > 0.98\) than a linear one \(r^2 < 0.94\). This exception may have arisen because there was a greater variation in temperature in this treatment, with an early period of low temperature followed by a phase of rising temperatures. Consequently, the rates of leaf appearance and of leaf initiation and the mean soil temperature, at a depth of 5 cm, for the period of study were re-calculated after weighting records according to the duration of each sub-phase.

Significant relationships were attained in the response of leaf initiation \(P < 0.05, r^2 > 0.82\) and leaf appearance rates \(P < 0.05, r^2 > 0.81\), to mean soil temperature, at a depth of 5 cm, from sowing to tassel initiation, for all hybrids, but some experiments did not give a good fit in the general regression analysis. In spite of the similar temperature range in expts 2, 3 and 4, leaf initiation rate of all hybrids was lower in expt 3 (0.32 leaves d\(^{-1}\)) than in expt 2 (0.49 leaves d\(^{-1}\)), and leaf appearance rate was lower in expts 3 (0.19 leaves d\(^{-1}\)) and 4 (0.23 leaves d\(^{-1}\)) than in expt 2 (0.31 leaves d\(^{-1}\)). This may have been caused by the lower PAR input in expts 3 and 4. Exclusion of these data (expt 3 for leaf initiation rate, and expts 3 and 4 for leaf appearance rate) improved the fit of the linear relationships \(r^2 > 0.94\) in the response to temperature of leaf initiation rate \(P < 0.01\) and leaf appearance rate \(P < 0.05\). Significant differences \(P < 0.05\) were detected among hybrids. Plastochron values (Table 2) ranged between 24-3 and 36-4°Cd, while \(T_b\) for leaf initiation ranged between 4-0 and 8-2°C. Genotypic differences in the response of leaf initiation rate to temperature grouped the hybrids into three sets: hybrids 33G26 and 36G12; hybrids 34K77, 34N16 and DK315; and the remaining genotypes. Phyllochron values (Table 3) ranged between 48-6 and 65-5°Cd, while \(T_b\) for leaf appearance varied between 2-9 and 5-0°C. The response of leaf appearance rate to temperature of hybrids 33G26 and 36G12 differed significantly \(P < 0.05\) from the fit obtained for most of the other genotypes.

Differences in the response to temperature of leaf initiation and leaf appearance rates were not related to final leaf number. For instance, hybrids 33G26 and 34N16 had a

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**RESULTS**

**Growing conditions**

Plants were exposed to contrasting growing conditions due to the wide range of sowing dates. Median soil temperature at a depth of 5 cm varied between 13.7 and 25.5°C (Fig. 1A) during the period of study (i.e. from three to nine appeared leaves). Median incident PAR in the same period ranged between 4.3 and 10.6 MJ m\(^{-2}\) d\(^{-1}\) (Fig. 1B).
### Table 2. Plastochron (°Cd), base temperature (°C) and regression coefficient ($r^2$) of the models between leaf initiation rate and soil temperature, at a depth of 5 cm, of 16 hybrids grown under five different environmental conditions in Argentina

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Plastochron* (°Cd)</th>
<th>Base temperature* (°C)</th>
<th>$r^2$</th>
<th>Significance†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrids with final leaf number &lt;17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANIOU 258</td>
<td>31.7 (2.1)</td>
<td>4.0 (1.0)</td>
<td>0.99</td>
<td>b</td>
</tr>
<tr>
<td>MONUMENTAL</td>
<td>26.0 (1.4)</td>
<td>6.7 (0.7)</td>
<td>0.99</td>
<td>b</td>
</tr>
<tr>
<td>39R52</td>
<td>25.7 (2.7)</td>
<td>6.4 (1.3)</td>
<td>0.97</td>
<td>bcd</td>
</tr>
<tr>
<td>39A26</td>
<td>26.7 (2.5)</td>
<td>6.6 (1.2)</td>
<td>0.97</td>
<td>bcd</td>
</tr>
<tr>
<td>Hybrids with final leaf number 17–19.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36G12</td>
<td>36.4 (4.0)</td>
<td>4.8 (1.6)</td>
<td>0.97</td>
<td>a</td>
</tr>
<tr>
<td>DK315</td>
<td>25.8 (2.4)</td>
<td>5.5 (1.3)</td>
<td>0.98</td>
<td>e</td>
</tr>
<tr>
<td>DK312</td>
<td>27.4 (4.0)</td>
<td>5.5 (2.0)</td>
<td>0.94</td>
<td>bcd</td>
</tr>
<tr>
<td>34N16</td>
<td>24.3 (1.5)</td>
<td>8.2 (0.7)</td>
<td>0.99</td>
<td>cd</td>
</tr>
<tr>
<td>33G26</td>
<td>35.5 (3.9)</td>
<td>5.4 (1.5)</td>
<td>0.97</td>
<td>a</td>
</tr>
<tr>
<td>34K77</td>
<td>24.3 (1.4)</td>
<td>8.1 (0.7)</td>
<td>0.99</td>
<td>d</td>
</tr>
<tr>
<td>Hybrids with final leaf number &gt;19.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DK664MG</td>
<td>26.5 (2.0)</td>
<td>6.7 (1.0)</td>
<td>0.98</td>
<td>bcd</td>
</tr>
<tr>
<td>DK615</td>
<td>26.1 (2.7)</td>
<td>7.0 (1.3)</td>
<td>0.97</td>
<td>bcd</td>
</tr>
<tr>
<td>34B15</td>
<td>29.6 (1.6)</td>
<td>5.7 (0.7)</td>
<td>0.99</td>
<td>bc</td>
</tr>
<tr>
<td>DK834</td>
<td>25.8 (2.6)</td>
<td>6.7 (1.3)</td>
<td>0.97</td>
<td>bcd</td>
</tr>
</tbody>
</table>

* s.e.m. values in parentheses.
† $n = 5$ for all hybrids.
†† Hybrids with the same letter did not differ in the simple regression models fitted to their data (P < 0.05).

### Table 3. Phyllochron (°Cd), base temperature (°C), and regression coefficient ($r^2$) of the models between leaf initiation rate and soil temperature, at a depth of 5 cm, of 16 hybrids grown under four different environmental conditions in Argentina

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phyllochron* (°Cd)</th>
<th>Base temperature* (°C)</th>
<th>$r^2$</th>
<th>Significance†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrids with final leaf number &lt;17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANIOU 258</td>
<td>55.0 (3-3)</td>
<td>3.8 (0-9)</td>
<td>0.99</td>
<td>bc</td>
</tr>
<tr>
<td>MONUMENTAL</td>
<td>53.1 (8-3)</td>
<td>3.7 (2-5)</td>
<td>0.95</td>
<td>bcd</td>
</tr>
<tr>
<td>39R52</td>
<td>52.4 (2-3)</td>
<td>3.4 (0-7)</td>
<td>0.99</td>
<td>d</td>
</tr>
<tr>
<td>39A26</td>
<td>48.6 (3-5)</td>
<td>5.0 (1-0)</td>
<td>0.99</td>
<td>bcd</td>
</tr>
<tr>
<td>Hybrids with final leaf number 17–19.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36G12</td>
<td>60.7 (1-4)</td>
<td>2.9 (0-4)</td>
<td>0.99</td>
<td>b</td>
</tr>
<tr>
<td>DK315</td>
<td>51.2 (8-4)</td>
<td>3.5 (2-6)</td>
<td>0.95</td>
<td>bcd</td>
</tr>
<tr>
<td>DK312</td>
<td>51.4 (7-5)</td>
<td>3.4 (2-4)</td>
<td>0.96</td>
<td>cd</td>
</tr>
<tr>
<td>34N16</td>
<td>50.0 (3-9)</td>
<td>5.0 (1-1)</td>
<td>0.99</td>
<td>bcd</td>
</tr>
<tr>
<td>33G26</td>
<td>65.5 (5-2)</td>
<td>3.1 (1-3)</td>
<td>0.99</td>
<td>a</td>
</tr>
<tr>
<td>34K77</td>
<td>51.3 (4-8)</td>
<td>5.0 (1-4)</td>
<td>0.98</td>
<td>abc</td>
</tr>
<tr>
<td>Hybrids with final leaf number &gt;19.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DK664MG</td>
<td>49.4 (4-6)</td>
<td>4.8 (1-4)</td>
<td>0.98</td>
<td>bcd</td>
</tr>
<tr>
<td>DK615</td>
<td>50.1 (8-6)</td>
<td>4.4 (2-6)</td>
<td>0.94</td>
<td>bcd</td>
</tr>
<tr>
<td>34B15</td>
<td>49.9 (2-5)</td>
<td>4.5 (0-8)</td>
<td>0.99</td>
<td>cd</td>
</tr>
<tr>
<td>DK682</td>
<td>49.3 (4-9)</td>
<td>4.5 (1-5)</td>
<td>0.98</td>
<td>bcd</td>
</tr>
<tr>
<td>DK752</td>
<td>49.8 (7-0)</td>
<td>4.6 (2-1)</td>
<td>0.96</td>
<td>bcd</td>
</tr>
<tr>
<td>DK834</td>
<td>51.6 (4-0)</td>
<td>3.6 (1-2)</td>
<td>0.99</td>
<td>cd</td>
</tr>
</tbody>
</table>

* s.e.m. values in parentheses.
† $n = 4$ for all hybrids.
†† Hybrids with the same letter did not differ in the simple regression models fitted to their data (P < 0.05).

Fig. 2. Response of leaf initiation rate (A) and leaf appearance rate (B) to temperature, at a depth of 5 cm, for two contrasting hybrids of similar final leaf number, grown under six different environmental conditions. Continuous and dashed lines represent the fitted functions, whereas dotted lines represent the extrapolation of the functions to $y = 0$ (for details see Tables 2 and 3). The circled data points were excluded from the analyses (see text).

Co-ordination model

Seeds of all hybrids had five leaf primordia plus the coleoptile in the embryo, and 18 new leaf primordia were initiated between sowing and seedling emergence (mean across hybrids and sowing dates). There was a strong relationship ($r^2 = 0.96, P < 0.0001$) between the number of initiated leaves and the number of appeared leaves for the period between seedling emergence and tassel initiation (Fig. 3). The co-ordination model described this relationship well for all hybrids and environments, and indicated a breakpoint at approx. 3.0 appeared leaves (i.e. 8–0 initiated leaf primordia). Leaf appearance before the breakpoint was relatively faster (2.4 appeared leaves per initiated
primordia) than after it (0.63 appeared leaves per initiated primordia).

All genotypes and environments included in the analysis of co-ordination behaved similarly. Among hybrids, the main component of the mean squared deviation was the lack of correlation weighted by the standard deviations (91.2%), although correlation coefficients were higher than 0.97), followed by the squared difference between standard deviations (7.2%) and the squared bias (1.6%; Table 4). The coefficient of variation (CV) for hybrids ranged between 7.7 and 13.9% Among environments, the main component of the mean squared deviation was also the lack of correlation weighted by the standard deviations (92.3%), correlation coefficients again were higher than 0.97), followed by the squared difference between standard deviations (4.2%) and the squared bias (3.5%), and the CV varied between 7.9% and 13.2% (Table 5). In other words, the differences between the predicted and observed number of appeared leaves among hybrids and environments were related to the pattern of fluctuation among measurements (i.e., lack of correlation weighted by the standard deviations). Such differences were not related to the magnitude of the fluctuation (i.e., squared difference between standard deviations), nor to the differences among means (i.e., squared bias).

The observed number of appeared leaves was predicted well by the co-ordination model (Fig. 4), as reflected in low CV values. For the first data set used for co-ordination model validation (genotypes B73, Mo17 and B73 × Mo17), the mean component of the mean squared deviation was the lack of correlation weighted by the standard deviations (86.3%), despite the fact that correlation coefficients were higher than 0.97), followed by the squared bias (9.8%) and then the squared difference between standard deviations (3.9%). The root mean square error (RMSE) was 0.47 appeared leaves for B73, 0.46 appeared leaves for Mo17, and 0.36 appeared leaves for B73 × Mo17. These values represented a CV of 7.6%, 9.0%, and 5.6%, respectively. For the second evaluated data set (source: Tollenaar and Hunter, 1983), the RMSE was 0.32 appeared leaves for exp 1 (CV = 4.3%), 0.35 appeared leaves for exp 3 (CV = 4.9%) and 0.12 appeared leaves for exp 2 (CV = 1.4%), and the main source of variation was the squared bias. This trend in the second data set led to an underestimation of the observed data, which was slightly larger for expts 1 and 3.
DISCUSSION

Plastochron and phyllochron

This work is the first to present, based on frequent observations of leaf primordia differentiation in the apex, an indication of the genotypic variability in the response of leaf initiation rate to temperature in currently used commercial maize hybrids (Table 2). For most tested hybrids, plastochron values were larger than 20.6 °C and \( T_b \) was slightly smaller than 7.3 °C (the only values in the literature obtained from direct apex observation; Hesketh and Warrington, 1989). In the present study, the plastochron of hybrids 33G26 and 36G12 was 30 % larger than that of hybrids DK312 and DK315, in spite of similar values of \( T_b \). Coligado and Brown (1975) reported genotypic differences in leaf initiation rate at high temperatures (e.g. 30 °C), but their results were based on a simple estimation of the rate as the quotient between total leaf number and the number of days up to tassel initiation, and they did not consider leaf primordia present in the embryo or carry out frequent samplings for apex observation. On the other hand, detailed studies on the maize mutant tel (Veit et al., 1998) indicated that its leaf initiation rate was twice that of the wild type, but its use in breeding is limited because the gene responsible for this response also modifies leaf organization around the stem (phyllotaxis) and promotes other major disorders in the plant.

The present results indicate that leaf initiation rate might be affected by growing conditions, being reduced 43 % in expt 3 as compared with expt 2. This response suggests a possible effect of PAR input, since the temperature regime was very similar in both experiments. Nevertheless, further research is needed on this topic to demonstrate clearly the effects of irradiance in leaf initiation rate.

This paper also provides a first report of genotypic differences in the response of maize leaf appearance rate to temperature based on the simultaneous estimation of phyllochron and \( T_b \), under field conditions (Table 3). Hybrids 33G26 and 36G12 had phyllochron values that are 23 % larger than those of most of the evaluated genotypes, whereas the relevant \( T_b \) did not vary markedly. Tollenaar et al. (1979) reported differences in leaf appearance rate of 10 % among hybrids growing at 10 and 35 °C, and similar results (differences of 15 % in the rate) were obtained by Tollenaar et al. (1984) for plants growing at 19 and 29 °C. By contrast, Giauffret et al. (1995) detected no differences in phylochron for hybrids but reported variation for this parameter and for \( T_b \) in inbred lines, although they found a weak relationship between leaf appearance rate and soil temperature at a depth of 10 cm (\( r^2 = 0.53 \)).

The phylochron values obtained here (Table 3) were larger than values from experiments under controlled conditions, whereas the \( T_b \) values were smaller. For instance, the fit by linear regression of the data of Tollenaar et al. (1979) by Ritchie and NeSmith (1991) yielded an estimated phylochron of 39 °Cd and a \( T_b \) of 8 °C, although the authors pointed out the large variation in these parameters that could be caused by slight differences in source data and by the regression-and-extrapolation approach. An analysis of influential points (Neter et al., 1996), using data from all hybrids in this experiment, revealed that records from expt 5 belonged to this category (\( R_{\text{student}} = -1.9 \)), because they promoted a decline in the slope (\( \Delta F_{\text{Beta}} y = -4.3 \)) and an increase in the ordinate (\( \Delta F_{\text{Beta}} x = 3.4 \)) that together resulted in a decline in \( T_b \). Two reasons may account for the variation from the general trend in the data from expt 5: the use of apex instead of air temperature and the low irradiance. In relation to the former, Vinocur and Ritchie (2001) estimated a difference in apex temperature between 4 and 10 °C for data obtained by Tollenaar et al. (1979), depending on the air temperature regime, and demonstrated...
that this source of error could be eliminated by using soil temperature at a depth of 5 cm (i.e. a good surrogate of apex temperature). In the present experiment, however, differences in temperature between soil at a depth of 5 cm (the data used) and the air were minimal (approx. −0.2 °C in expt 1 pre-germinated, 0-8 °C in expt 1 non-pre-germinated, 1-7 °C in expt 2, and −0.5 °C in expt 5). Correction by this factor, therefore, did not change the pattern of differences in phyllochron and $T_0$ values among genotypes. With respect to low irradiance effects, shading experiments (Birch et al., 1998) indicated a decline of between 2 and 4 °Cd ($T_0$ of 8 °C) per megajoule of reduction in daily PAR, and this response increased under high temperature. In the present work, leaf appearance rate was lower in expts 3 (−48-9 %) and 4 (−27-3 %) than in expt 2, in agreement with the reduction in PAR (−70 % in expt 3 and −30 % in expt 4 as compared with expt 2) but not in temperature of these experiments. On the other hand, high irradiance was accompanied by high temperature in expt 5, and yielded a photothermal combination that resulted in an increase in leaf appearance rate of only 10 % compared with expt 2, for which a high irradiance regime was accompanied by reduced temperatures (Fig. 1).

Finally, the present results on genotypic differences in plastochron and phyllochron did not suggest a relationship of these traits with total leaf number, in contrast to reports by Eik and Hanway (1965). These authors measured a higher leaf appearance rate in early maturing hybrids, a trend not confirmed by Tollenaar et al. (1984) or by the present work. Consequently, the presence of genotypic variability for these attributes in all maturity groups provides scope for the search of enhanced developmental rates as secondary traits in breeding programmes independently of the target environment. This strategy would be helpful for advanced silking date with no reduction in leaf number (Dijak et al., 1999).

**Co-ordination model**

In wheat, a crop extensively studied, the strong co-ordination found among developmental processes—leaf initiation, spikelet differentiation, floret differentiation, leaf appearance and stem extension—contributes to convergent development (i.e. the fully developed ears emerge a few days after the end of the flag leaf growth; Hay and Kirby, 1991). In maize, the convergence between tassel emergence and the expansion of the flag leaf has been reported for a wide range of cultivars and photoperiods (Kiniry et al., 1983). This paper focuses on the possible co-ordination between the processes of leaf initiation and leaf appearance. Several hypotheses have been proposed to understand the association between leaf initiation and leaf appearance, but the responsible processes still remain unclear. Kirby (1990) hypothesized that co-ordination could be mediated by a physical restriction as well as by hormones produced in leaves undergoing expansion. On the other hand, Sadras and Villalobos (1993) proposed that both initiation and expansion respond in a similar way to contrasting environments.

This paper is the first report of the existence of a strong relationship between leaf initiation and leaf appearance processes in maize, from seedling emergence to tassel initiation. The co-ordination model was not affected by genotype (some of which differed significantly in the response to temperature of leaf initiation and leaf appearance rates) or by the large variation in the photothermal environment introduced by contrasting sowing dates (e.g. similar temperature regime of expts 2, 3 and 4, whereas the input of PAR varied). These findings are in agreement with previous research on rice (Nemoto et al., 1995), wheat (Kirby, 1990), pea (Turc and Lecoeur, 1997) and sunflower (Sadras and Villalobos, 1993), which also included different genotypes and photothermal conditions. Moreover, the co-ordination model fitted here was successfully validated using an independent data set that comprised two inbred lines and their hybrid, each grown in two environments. This co-ordination model was able to predict accurately the mean number of appeared leaves (i.e. squared bias), the magnitude of the variation among data (i.e. squared difference between standard deviations), and to a lesser extent the pattern of the fluctuations among data (i.e. lack of correlation weighted by the standard deviations), although the correlation coefficient was always high (>0.97).

A remarkable aspect of the above-mentioned co-ordination model was the biphasic relationship between initiated leaf primordia and appeared leaves. Plants had, on average, 6-8 initiated primordia in the apex at seedling emergence, which is very close to the value of 6-3 reported by Warrington and Kanemasu (1983) using a sowing depth shallower than in the present research. Moreover, the breakpoint of the co-ordination model (approx. 2-8 appeared leaves) matched the stage of transition between the heterotrophic phase, when growth depends on seed reserves, and the autotrophic phase, when it starts to depend on photosynthesis (Cooper and MacDonald, 1970). The faster developmental rate of the first phase (i.e. the seedling stage) is in agreement with evidence from rice (Nemoto et al., 1995) and contrasts with findings from two dicots, sunflower (Sadras and Villalobos, 1993) and pea (Turc and Lecoeur, 1997). The rate of increase in the second phase (0-63 appeared leaves per initiated primordia in the apex) is indicative of the relationship between the phyllochron and the plastochron, providing that a common $T_0$ exists for both attributes. The only information on this topic available in the literature indicates a rate of 0-69 for the second phase (Hesketh and Warrington, 1989). This rate was obtained with a plastochron value of 20-6 °Cd ($T_0$ of 7-3 °C) and a phyllochron value of 30-0 °Cd ($T_0$ of 8-9 °C), the latter obtained for the period between 3 and 12 appeared leaves. On the basis of the consistency in co-ordination between the plastochron and the phyllochron, and the lack of genotypic variability for this attribute, an easily quantified trait such as leaf appearance rate could be used for breeding purposes (e.g. the development of hybrids with faster leaf initiation rate), and in the study of the genetic control of plastochron. This strategy has been adopted recently in rice, for which mutants of the gene plastochron1 have been identified on the basis of their higher leaf appearance rate. Expression of the gene increases leaf initiation rate 2-fold compared with the wild type, and is limited to primordia undergoing development (Miyoshi et al., 2004).
Finally, the present co-ordination model was validated successfully at tassel initiation with an independent data set. Tollenaar and Hunter (1983) found a near constant relationship of 0.5 between the number of appeared leaves and total leaf number at tassel initiation, but they used only one hybrid and did not establish the general applicability of this ratio. Based on a wide range of genotypes, the present work has demonstrated that the relationship is not constant for the period between seedling emergence and tassel initiation, and that it depends on the number of appeared leaves at which tassel initiation occurs. The co-ordination model predicts a ratio of 0.5 at around 8-2 appeared leaves, approximately the same stage as in Tollenaar and Hunter (1983). Therefore, it could be used as an a posteriori non-destructive method for the estimation of tassel initiation, based on frequent observations of appeared leaves around the time of apex transition, and knowledge of final leaf number. This would allow for a more efficient use of resources in studies of plant development under controlled conditions, where the space available in growing chambers is usually limited, and also would help in the study of the early establishment of hierarchies among plants during the crop growth cycle, which is a critical issue in the analysis of maize tolerance to increase stand density (Maddonnii and Otegui, 2004).

In conclusion, the existence of genotypic variability within currently used maize hybrids in the response to temperature of leaf initiation rate and leaf appearance rate has been demonstrated. In spite of evidence of major effects of genotype on plastochron and phyllochron, these results from field experiments differ from research performed under controlled conditions, especially in allowing analysis at temperatures closer to $T_b$. The results for maize suggest that co-ordination between development (i.e. organogenesis) and growth (i.e. expansion) is a common feature of many crops, and research on the processes responsible of this co-ordination will be of value in understanding leaf development in plants. This paper has also shown the strong interdependence between leaf initiation and leaf appearance, which determined the fit of a single co-ordination model applicable to the whole data set during the period between seedling emergence and tassel initiation. The co-ordination model was successfully validated for different independent data sets, one of which was obtained from the literature, supporting its use in crop simulation models for improving the estimation of canopy development.

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LITERATURE CITED


