Leaf area development in maize hybrids of different stay-green rating

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Abstract

In maize, soil nitrogen (N) influences the rate of leaf expansion and consequently the amount of light intercepted. In this study, the rate of leaf appearance and green area development of four 'stay-green' (sgr) maize hybrids (P39K38 (sgr 6), P38V12 (sgr 7), P38F70 (sgr 8) and P38G43 (sgr 9)) were examined in response to N fertiliser. Crops were grown with no fertiliser N or 270 kg N ha^{-1}. The rate of leaf appearance was consistent at around 34.3 ^{o}Cd per leaf tip and around 50.2 ^{o}Cd per ligule with no difference among cultivars. A maximum mean green area index (GAI) of 5.3 was achieved in the N fertilised crops compared with 3.9 for the non-fertilised crops. The maximum GAI ranged from 5.0 in P38F70 (sgr 8) to 6.0 in P38V12 (sgr 7) with 5.2 for P38G43 (sgr 9) and 5.1 for P39K38 (sgr 6). At maximum GAI, P38F70 (sgr 8) had 16.8 leaves compared with 15.9 for both P39K38 (sgr 6) and P38G43 (sgr 9) while P38V12 (sgr 7) had 15.2. Given that leaves appeared at a constant rate in all hybrids, the higher GAI was attributed to differences in the rate of leaf expansion. These differences may affect total radiation interception and consequently biomass yield.

Additional keywords: *Zea mays*, stay-green maize, green area index, leaf appearance

Introduction

Under non-limiting conditions, temperature has a greater influence on leaf area development in maize (*Zea mays* L.) than radiation or nutrient availability (Wilson *et al.*, 1995). The main influence of temperature is on the rate of leaf tip appearance (Stone *et al.*, 1999) and the duration of leaf expansion (Warrington and Kanemasu, 1983b). Stable rates of leaf appearance against mean temperature can be derived (Muchow and Carberry, 1989) because leaf initials appear at a constant rate from emergence until the onset of reproductive growth (Warrington and Kanemasu, 1983a). Available evidence suggests a linear response between the rate of development and mean temperature within given temperature limits (Warrington and Kanemasu, 1983b; Muchow *et al.*, 1990). This linear increase in leaf initiation with increased temperature (Warrington and Kanemasu, 1983a), makes the time based concept of thermal time (Tt) an appropriate way of characterising leaf appearance rates

(Muchow and Carberry, 1989). In a number of plant species a constant number of leaves appear per unit of thermal time (Hay and Porter, 2006).

The ontogenic development of leaf area consists of three important stages. The initial stage of leaf initiation and expansion, the fully expanded and active photosynthetic stage and finally leaf senescence (Dale and Milthorpe, 1983). Maximum biomass production depends on the full utilisation of the period between full canopy expansion and the onset of senescence. During plant senescence proteins and nucleic acids are broken down (Thomas and Stoddart, 1980) leading to a loss of chlorophyll and a decline in photosynthetic activity (Leopold, 1980). A number of plant species, including maize and sorghum (*Sorghum bicolour* L. Moench), exhibit a characteristic known as 'stay-green' where the normal process of senescence is delayed (Borrell and Hammer, 2000). It is manifested through a delayed onset or a reduced rate of leaf senescence (Thomas and Howarth, 2000). By delaying senescence or reducing its rate, hybrids with 'stay-green' characters may have the opportunity to intercept more solar radiation and hence potentially accumulate more dry matter (DM). In maize, the greatest biomass increase occurs after silking (Muchow *et al.*, 1990) when leaf area is at its maximum, and when the pool of reserves available for partitioning to grain has a direct influence on potential biomass.

In New Zealand, maize is used as a rotation crop with the potential for high DM production (Wilson *et al.*, 1994) and is primarily used as a high quality feed in dairy systems. Previous studies have quantified the relationship between temperature and developmental events in maize in terms of accumulated thermal units (Wilson *et al*., 1995; Fletcher *et al.*, 2008). However, data on maize hybrids that differ in their 'stay-green' rating are limited. In this study the relationship between accumulated Tt and leaf appearance rate was determined for four maize cultivars with 'stay-green' ratings ranging from 6-9. A score of 9 is assigned to the cultivar with the highest number of non-senescent leaves below the ear three weeks prior to silage harvest maturity. This is followed by an investigation of the influence of N on the rate of leaf appearance and the development of green leaf area.

Materials and Methods

Experimental design

The experiment was a split plot in a randomised complete block with two irrigation treatments (dry or fully irrigated) as main plots. Two rates of N (0 and 270 kg N ha^{-1}) and four maize hybrids (P39K38 (sgr 6), P38V12 (sgr 7), P38F70 (sgr 8) and P38G43 (sgr 9)) were then arranged in fully randomised sub-plots and replicated three times. Each plot measured 4.9 x 10 m with 0.7 m between maize rows and 0.15 m, within rows.

Cultural practices

The experiment was sown on 24 October 2008 into a Typic Immature Pallic Soil (Hewitt, 1998) with 0.4-1.0 m silt loam overlying gravel that had previously been sown in oats (*Avena sativa* L.) (2005 and 2008) and consecutive crops of wheat (*Triticum aestivum* L.) (2006 and 2007). Soil tests showed a pH of 6.0, an Olsen P level of

14 mg l^{-1} and 74 kg available N to 150 mm. Two seeds were sown per hole using a jab planter and thinned to one plant $hole^{-1}$ three weeks later. A deep N analysis taken to 1.0 m indicated a mineral N content of 44 kg N ha^{-1}. During land preparation, 560 kg ha^{-1} of 20% Potash Super, containing 7.4% P, 10% K, 8.6% S and 16% Ca was applied. A soil test four weeks after emergence showed that the Olsen P level had risen to 33 mg l^{-1}.

A pre-emergence application of atrazine (Nu-Traize 900 DF, 900 g kg $a.i^{-1}$) at 1.5 l ha^{-1} was used to control broad leaf weeds.

Two weeks after sowing, 50% of the plants had emerged. Ten days later five plants were selected for non-destructive sampling and tagged. After hand thinning the mean population was 9.25 plants m^{-2}. Additional N for the fertilised treatment was provided as urea (46% N) and was broadcast in two applications each of 135 kg N ha^{-1} at 16 and 37 days after emergence (DAE). A light overhead sprinkler irrigation of 10 mm followed each N application to dissolve the urea.

Measurements

During vegetative growth, leaf appearance was monitored every 3-4 days by recording the number of emerged leaf tips and fully expanded leaves. A fully expanded leaf and an emerged leaf tip were recorded as defined by Muchow and Carberry (1989). Green area increase was measured every 14 days whenever the weather allowed using a Sun Scan (Delta-T Devices, Cambridge-England). Water extraction from the profile was monitored weekly using Time Domain Reflectometry (TDR) (Trase System 1 Model 6050 X1) and a Neutron Probe (NMM Model 3300) at 0.1 m intervals to a depth of 0.4-1.0 m dependent on the depth to gravel. Irrigation water was applied to restore the moisture depleted from the top 0.2 m of the soil profile to near field capacity when the total available water content dropped to 15%. Air temperatures were logged hourly with three thermistors located in the central plot of each replicate.

Calculations and data analysis

This paper only reports the data from the fully irrigated treatment. Daily thermal units were accumulated after emergence from air temperatures using the modified sine curve method (Fletcher, 2005) with a base temperature of 0 ^{o}C (Wilson *et al.*, 1995) and an optimum temperature of 34 ^{o}C (Muchow and Carberry, 1989). Usually the final three leaves appear at a faster rate because of their smaller size (Warrington and Kanemasu, 1983b) and this distorts the linear relationship between leaf appearance rate and Tt. In this study a two-stage linear regression was fitted to the relationship between leaf appearance and Tt using a series of dummy variables (Draper and Smith, 1998). The maximum R^2 was used as the criterion to partition data points to the two line segments (Fletcher *et al.*, 2008). Green area index (GAI) data were plotted as functions of time.

Statistical analysis was done with Genstat 11, Release 11.1 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2008). All variates were analysed using ANOVA procedures in a randomised complete block design structure and the least significant difference ($P<0.05$) was used to separate means.

Results

There were no interactions between hybrids and N treatment. Therefore main effects of hybrid and N treatments only are reported.

Leaf appearance rate

The relationship between the number of visible leaf tips and accumulated thermal time above a base temperature of 0 ^oC was linear. Nitrogen did not influence ($P<0.49$) the rate of leaf tip appearance (reciprocal of the coefficient of the linear regression). The phyllochrons were 35 and 33.9 ^oCd leaf tip^{-1} for the 0 and 270 kg N ha^{-1} treatments, respectively, but were not significantly different.

Among hybrids, leaf tips appeared linearly with accumulated thermal time (Figure 1a). The phyllochrons ranged ($P<0.36$) from 33.3 ^oCd leaf tip^{-1} in P38F70 (sgr 8) to 35.2 ^oCd leaf tip^{-1} in P38G43 (sgr 9). The other cultivars were intermediate with 33.8 ^oCd for P39K38 (sgr 6) and 35.1 ^oCd for P38V12 (sgr 7). The appearance of fully expanded leaves over time was also unaffected by N fertiliser or cultivar and successive fully expanded leaves appeared after 50.0 ^oCd (Figure 1 b).

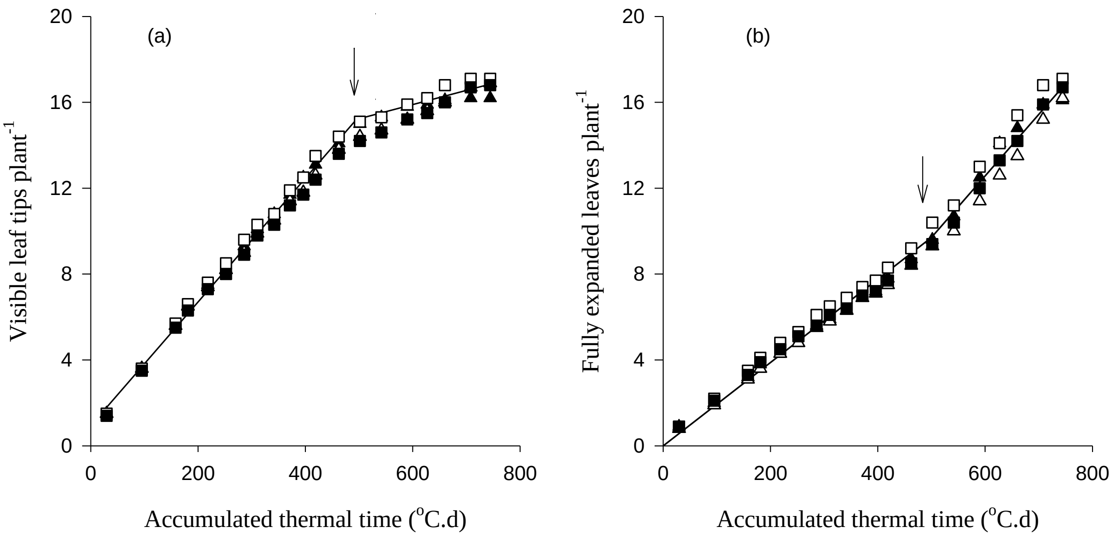

Figure 1: The Number of visible leaf tips (a) and fully expanded leaves (b) plant^{-1} against accumulated thermal time (T_b = 0 ^oC) for P39K38 (sgr 6) (▲) P38V12 (sgr 7) (△) P38F70 (sgr 8) (□) and P38G43 (sgr 9) (■) grown at Lincoln University in 2008-09. Fitted regressions were
(a) $Y = 0.0291x+1$ ($R^2 = 0.99$) (S.E = 0.156 leaf tips plant^{-1}) and
(b) $Y = 0.0200x$ ($R^2 = 0.99$), (S.E = 0.19 leaves plant^{-1}), respectively.
Solid lines indicate the two-stage linear regression of visible leaf tips and fully expanded leaves on Tt. Arrows indicate the point of inflection.

Green area index

The GAI increased slowly from emergence and was still less than 1.0 at 40 DAE (Figure 2). It then increased rapidly until it reached a maximum at 75 DAE. Nitrogen fertiliser increased (P<0.001) the rate of green leaf area development. Mean maximum GAI in treatments without N was 3.9 (Table 1) compared with 5.3 for those that received 270 kg N ha^{-1} (Table 1) and these remained above the critical GAI of 4.0 for about 70 days (Figure 2 b). Nitrogen therefore increased GAI duration, enabling fertilised crops to intercept solar radiation for longer.

Hybrid differences (P<0.05) were observed for maximum GAI which ranged from 3.2 in P38F70 (sgr 8) to 4.8 in P38V12 (sgr 7) with no added N (Figure 2 a). Hybrids with the highest maximum GAI tended to be those with the lowest 'stay-green' ratings. These hybrids either developed more leaves or had larger leaves. Table 1 shows that P38V12 (sgr 7) had the highest GAI, but fewer (P<0.001) leaves than other hybrids. Under low N, the only hybrids that reached critical GAI (P38V12 (sgr 7) and P39K38 (sgr 6)) were those with a low 'stay-green' rating.

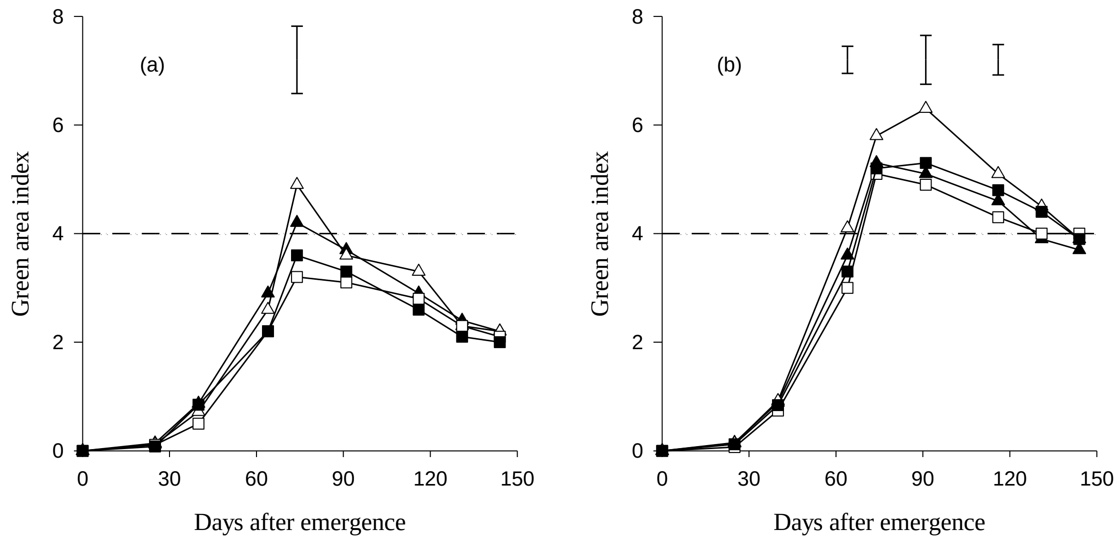

Figure 2: Green area index plotted against DAE for non-fertilised (a) and fertilised (b) crops of P39K38 (sgr 6) (▲), P38V12 (sgr 7) (△), P38F70 (sgr 8) (□) and P38G43 (sgr 9) (■) grown at Lincoln University in 2008-09. Each point is the mean of 3 replicates. Standard error = 0.296. The dashed line indicates the critical green area index.

Table 1: Maximum green area index and final number of leaves of four maize hybrids grown without or with 270 kg N ha^{-1} at Lincoln University in 2008-09.

	Maximum GAI		
Hybrid	270 kg N ha^{-1}	No N	Final number of leaves
P3K38 (sgr 6)	5.1b	4.2a	15.9b
P38V12 (sgr 7)	6.0a	4.8a	15.2c
P38F70 (sgr 8)	5.0b	3.2b	16.8a
P38G43 (sgr 9)	5.2b	3.4b	15.9b
Significance level	<0.001		< 0.001
LSD	0.59		0.66
CV (%)	15.6		4.9

Means followed by the same letter are not significantly different based on least significant tests at $\alpha = 0.05$.

Discussion

The conservative linear relationship between leaf tip appearance and accumulated Tt with or without additional N (Figure 1) is indicative of the constancy of initiation of phytomers at the stem apex (Hay and Porter, 2006). Successive leaf tips appeared after approximately 34.5 ^{o}Cd and each fully expanded leaf after 50 ^{o}Cd. These results are consistent with those of Vos *et al.* (2005) in two glasshouse experiments where the rates of N applied ranged from 0.5-6.0 g pot^{-1}. Both studies reported an average rate of leaf appearance of 50 ^{o}Cd $leaf^{-1}$. Muchow (1988) observed relatively small differences in leaf appearance rates in a field trial where N treatments ranged from 0 to 42 g N m^{-2}.

Linearity between accumulated Tt and leaf tip appearance (Figure 1a) or fully expanded leaf appearance (Figure 1b) rates among the hybrids was also observed. These results support the idea that the rate of leaf appearance (phyllochron) is a development process that is strongly related to temperature and is only influenced by fertility at extreme levels.

The influence of N was mainly on the temporal pattern of GAI development. Most maize crops intercept more than 90 % of the oncoming solar radiation when their GAI is greater than or equal to 4.0 (Birch *et al.*, 2003). A GAI of 4.0 was considered a critical minimum value for assessing growth and development responses in this study. Nitrogen deficiency limited green area increase in the non-fertilised crops with two of the hybrids not attaining the critical GAI of greater than or equal to 4.0 (Figure 2a). The non-fertilised crops therefore had GAI values below the critical GAI for most of the season which would reduce the total amount of solar radiation intercepted. In contrast, the fertilised crops were above the critical GAI for about 70 days (Figure 2b). Nitrogen deficiency reduces the rate of cell division and expansion (Dale and Milthorpe, 1983) by influencing the capacity of a plant to accumulate solutes resulting in restricted cell growth (Wolfe *et al.*, 1988). Because reductions in leaf area are permanent (Begg and Turner, 1976), there is a reduced potential to intercept solar radiation, specifically when the GAI is below critical values.

Figure 2 shows that both P39K38 and P38V12 had consistently higher GAIs under both N regimes. These hybrids had the two lowest ‘stay-green’ ratings of 6 and 7, respectively. It appears that under low N availability, both hybrids efficiently utilised available N to expand the leaf canopy. This was potentially achieved by mobilising available N from older leaves into younger and more actively growing leaves. Alternatively, these hybrids could also have partitioned more of the available N into leaf growth. According to Sinclair and Horie (1989), during early vegetative growth, plants must partition a certain proportion of available N between the demand for leaf growth and the maintenance of leaf nitrogen concentration. Greater partitioning to leaf growth enhances the development of leaf area (Vos *et al.*, 2005). Future analysis of leaf N concentrations will be used to examine the impact of ‘stay-green’ on nitrogen allocation.

Conclusion

The timing of developmental events during maize vegetative growth was unaffected by N fertiliser and maize hybrid. Temperature was a strong driver of development as defined by leaf tip appearance and rates of fully expanded leaf production. Nitrogen influenced the maximum GAI and GAI duration and there were differences among hybrids in their ability to accumulate GAI in both the low and high N treatments.

In most cases the hybrids P38V12 and P39K38, with low ‘stay-green’ rating developed a larger green leaf area than the other hybrids (P38F70 and P38G43). This may have occurred by mobilising N reserves to support leaf enlargement, but the rate of decline of leaf area was higher toward the end of grain filling. There appeared to be no relationship between ‘stay-green’ rating and phyllochron.

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References

Begg, J.E. and Turner, N.C. 1976. Crop water deficits. *Advances in Agronomy* 28: 161-217.

Birch, C.J., Vos, J. and van der Putten, P.E.L. 2003. Plant development and leaf area production in contrasting cultivars of maize grown in a cool temperate environment in the field. *European Journal of Agronomy* 19: 173-188.

Borrell, A.K. and Hammer, G.L. 2000. Nitrogen dynamics and the physiological basis of stay-green in sorghum. *Crop Science* 40: 1295-1307.

Dale, J.E. and Milthorpe, F.L. 1983. General features of the production and growth of leaves. pp. 151-178. *In*: The growth and functioning of leaves. Eds Dale, J.E. and Milthorpe, F.L. Cambridge University Press, Cambridge.

Draper, N.R. and Smith, H. 1998. Applied regression analysis. 3rd Edition. Wiley-Interscience, New York. 706 pp.

Fletcher, A.L. 2005. Understanding 'Challenger' sweet corn yield, quality and phenology responses to phosphorus. PhD Thesis. Lincoln University, Canterbury, New Zealand.

Fletcher, A.L., Moot, D.J. and Stone, P.J. 2008. Solar radiation interception and canopy expansion of sweet corn in response to phosphorus. *European Journal of Agronomy* 29: 80-87.

Hay, R. and Porter, J. 2006. The physiology of crop yield. 2nd Edition. Blackwell Publishing, Oxford. 314 pp.

Hewitt, A.E. 1998. New Zealand soil classification 2nd Edition. *Landcare Research Science Series No. 1*.

Leopold, A.C. 1980. Aging and senescence in plant development. pp. 1-11. *In*: Senescence in plants. Ed. Thimann, K.V. CRC Press, Boca Raton, Florida.

Muchow, R.C. 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment I. Leaf growth and leaf nitrogen. *Field Crops research* 18: 1-16.

Muchow, R.C. and Carberry, P.S. 1989. Environmental control of phenology and leaf growth in tropically adapted maize. *Field Crops Research* 20: 221-236.

Muchow, R.C., Sinclair, T.R. and Bennett, J.M. 1990. Temperature and solar radiation effects on potential maize yield across locations. *Agronomy Journal* 82: 338-343.

Sinclair, T.R. and Horie, T. 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: A review. *Crop Science* 29: 90-98.

Stone, P.J., Sorensen, I.B. and Jamieson, P.D. 1999. Effect of soil temperature on phenology, canopy development, biomass and yield of maize in a cool-temperate climate. *Field Crops Research* 63: 169-178.

Thomas, H. and Howarth, C.J. 2000. Five ways to stay green. *Journal of Experimental Botany* 51: 329-337.

Thomas, H. and Stoddart, J.L. 1980. Leaf senescence. *Annual Review of Plant Physiology* 31: 83-111.

Vos, J., van der Putten, P.E.L. and Birch, C.J. 2005. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthesis capacity in maize (*Zea mays* L.). *Field Crops Research* 93: 64-73.

Warrington, I.J. and Kanemasu, E.T. 1983a. Corn growth response to temperature and photoperiod I. Seedling emergence, tassel initiation, and anthesis. *Agronomy Journal* 75: 749-754.

Warrington, I.J. and Kanemasu, E.T. 1983b. Corn growth response to temperature and photoperiod II. Leaf-initiation and leaf-appearance rates. *Agronomy Journal* 75: 755-761.

Wilson, D.R., Johnstone, J.V. and Salinger, M.J. 1994. Maize production potential and climatic risk in the South Island of New Zealand. *New Zealand Journal of Crop and Horticultural Science* 22: 321-334.

Wilson, D.R., Muchow, R.C. and Murgatroyd, C.J. 1995. Model analysis of temperature and solar radiation limitations to maize potential productivity in a cool climate. *Fields Crops Research* 43: 1-18.

Wolfe, D.W., Henderson, D.W.; Hsiao, T.C. and Alvino, A. 1988. Interactive water and nitrogen effects on senescence of maize. I. Leaf area duration, nitrogen distribution, and yield. *Agronomy Journal* 80: 859-864.