DIAGNOSIS OF NITROGEN DEFICIENCY I1\; MAIZE AND THE INFLUENCE OF HYBRID AND PLANT DENSITY

Daniel T. Walters University of Nebraska, Lincoln. Nebraska

Abstract

The precise diagnosis of maize N status has proven to be a difficult task because of annual variation in internal N-use efficiency (kg grain kg⁻¹ N uptake) and temporal change in N concentration in plant tissue. Analysis of leaf N concentration (g N g^{-1} leaf) has not been a consistently successful N diagnostic because of temporal and hybrid differences in specific leaf weight (SLW=g leaf m⁻² leaf). An alternative N diagnostic, specific leaf nitrogen (g N m⁻² leaf), is unrelated to leaf mass. Maximum maize $CO₂$ assimilation rate has been reported at threshold whole plant SLNwp of 1.5 g N m⁻². Field experiments were conducted to compare the differences in leaf development, SLW and SLNwp in relation to yield for four irrigated maize hybrids under different levels of N stress. In a separate experiment the impact of population density on SLNwp was also studied. Although leaf area development varied among hybrids, relative yield was maximized when SLNwp \exists 1.5 g N m⁻² leaf regardless of environment or hybrid. Leaf area index (LAI= m^2 leaf m⁻² land) is an important variable governing total N need of the crop canopy and will vary as a function of population. **A** second diagnostic. specific leaf N per unit land area (SLNla = g leaf N $m²$ land area) and was found to be a more robust indicator of maize canopy status especially in predicting N need at early growth stages. Remote sensing of canopy N status will most likely be improved when sensing devices that adequately measure canopy LAI are coupled to algorithms that relate canopy reflectance with SLN.

Introduction

Nitrogen deficiency is dificult to determine because of the range in internal N use efficiency (kg main yieldkg **h'** uptake) that may occur due to a multitude of yield limiting factors that may **⁶** occur during a given growing season. Also there is a non-linear relationship between yield and N uptake because of decreasing internal N use efficiency as yields approach the yield potential of the crop. (Figure 1)

Figure 1 shows the relationship between maize yield and N uptake from a family of on-farm data collected across the North-Central USA from 1995-2000 (Cassman et al., 2000). The upper boundary line in Figure 1 represents the area of maximum N dilution, which occurs when N is the most limiting factor and internal N use efficiency is high. Below that upper boundary. there is usually some other yield limiting factor (water supply. pest etc.) and the amount of grain produced per unit of N uptake decreases.

However, total biomass yield vs. N content has been shown to be a tightly conserved relationship within C_4 (e.g. maize) and C_3 (e.g. soybean) species (Figure 2). This data of Greenwood et al. (1 990) covers a wide range of species and environments and suggests hvo things. The first is that the relationship between *total maximum biomass* yield and N uptake is tightly conserved and second, as the plant accumulates biomass, N becomes diluted and so the critical boundary of N sufficiency is a "floating target" governed by the relationships depicted in Figure 1. Therefore, the amount of N uptake that will translate to econornic yield optimum depends upon reaching the critical N concentration depicted by Greenwood et al. and reducing factors that contribute to poor internal N use efficiency (Cassman et al. 2003). improvement in N-use efficiency will rely on real-time assessment of N sufficiency.

Figure 1. Relationship between maize grain yield and plant-N accumulation in aboveground biomass at physiological maturity (Cassman et al. 2000).

Figure 2. The relationship between total dry matter yield and biomass N concentration for a wide range of C_3 and C_4 crops (Greenwood et al., 1990).

According to Greenwood et al., diagnosis of maize N status would seem to require analysis of total N uptake and biomass to determine if the critical N concentration has been attained. However, optimum photosynthetic capacity is reached when N is optimally distributed on an *areal* leaf basis. Although leaf N concentration is closely related to chlorophyll content, changes

in specific leaf weight as the plant develops rcsults in stage specific correlation between leaf greenness (Fig. 3). If spectral analysis of leaf N status is to be successful, more physiologically relevant N diagnostics are needed. It has been shown that the maize canopy leaf nitrogen distribution at which $CO₂$ assimilation is maximized is at 1.5 g N m-2 leaf (Muchow and Sinclair. 1994: Lemaire et al. 1997). It would seem that with these more definitive and physiologically based N diagnostics, one might be able to estimate the optimum plant N concentration as a function of changing biomass growth, developmental conditions. hybrids and population density differences.

Figure 3. Relationship between leaf chlorophyll and (A) specific leaf nitrogen or (B) leaf N concentration at different stages of maize development. (Data from nine site years and six hybrids in NE.)

Approach

Here I will report from two separate experiments. In the first experiment, a complete factorial, randomized complete design was conducted at two sites in Nebraska to study the relationship between nitrogen supply and plant diagnostic criteria across four high yielding maize hybrids. Four hybrids were chosen for the study based upon their tolerance to insect and disease stress as well as historic yield performance. The four hybrids chosen were: Pioneer 33A14(Bt), Pioneer 33R88 (Bt). DeKalb 632 and Golden Harvest 2581(Bt). All hybrids were 114d RM. Two irrigated sites were chosen: Clay Center, NE (soil =Hasting sil, previous crop=maize) and Mead, NE (soil=Sharpsburg sicl. previous crop=soybean). These sites were approximately 100 miles from each other east to west. At each site. a factorial combination of N fertilizer rate (0. 80. 160 and 240 kg N/ ha) and hybrid were planted to achieve a final population of 70,000 pl/ha (28,000

pl/acre). The following plant measurements were made at five specific growth stages: (V8. V15, R1. R4 and R6) from $1m^*$ or $12m^+$ of row:

- 1. LAI^{*}: Leaf area index $(m^2 \text{ leaf } m^{-2} \text{ land area})$
- 2. SLW*: Specific leaf weight (g leaf m-2 leaf area)
- 3. SLNwp^{*}: Specific leaf N of whole plant (g N m-2 leaf)
- 4. LNla^{*}: SLN on a land area basis (g leaf N m-2 land area)
- 5. **TN*** i: Total N uptake gNm-2
- 6. GY \dagger : Final grain yield at R6
- 7. SY[†]: Final stover yield at R6

These parameters were subject to analysis of variance to determine the response to nitrogen rate as well as hybrid and N x hybrid interaction effects. Relative yield was also regressed against leaf and plant N diagnostics to determine the thresh-hold critical level of these diagnostics for optimum yield prediction. Total N uptake at maximum biomass accumulation at each of the sampled growth stages was regressed against total plant N concentration and compared to the published Greenwood et al. (1970) relationship.

In a second experiment conducted in Lincoln, NE, the effect of population on leaf and plant N diagnostics was also examined. A 3x2 factorial experiment was conducted in a split-plot RCB three plant populations (76 **(30),** 94 (37), 112 (44) pl/ha(a)) **as** the main-plot and two levels of nutrient management (M1: recommended fertilizer rates-138 kgN/ha, M2: intensive nutrient management-298 kgN/ha) as the sub-plot. Maize hybrid was Pioneer 33A14(Bt). All maize followed a previous crop of soybean and was irrigated. Plants were sampled at three growth stages (V6, V12 and VT) and measured for LAI, SLNwp, SLNdl and SLNla as in the first experiment. Grain yield and total N uptake was measured at R6 stage from 18m of row.

Summary

Experiment 1 (EIybrid x N rate)

Differences in hybrid performance were observed with respect to site and previous crop. Although there were no differences in yield at Mead, NE, hybrids P-33R88 and GH-2581 were reduced at Clay Center (Fig.4) due to lower harvest index and higher barren stalk percentage. respectively. Final total leaf area, however, showed greater variance at Mead especially with no supplemental N fertilization. Table 1 shows how specific leaf weight changes with N status and stage. These changes add to the variance observed in Fig.3. There were no hybrid effects observed for SLNwp and a small but significant hybrid difference in LNla in direct proportion to observed changes in LAI. This suggests a compensation in N distribution in leaf as a function of hybrid difference in LAI. SLNwp declined with increase in leaf biomass and this decline was greatest where plants were under N stress. At the same time, LNla increased in proportion to yield response to applied N. At Mead, LNla declined during reproductive development but did not at Clay Center. This suggests a critical SLNwp and LNla is physiologically important as only non-N responsive plots showed this trend (Fig. 5).

Figure 6 shows the capacity of SLNwp in predicting final relative yield. SLNwp was a poor predictor of final yield performance when measured at the V8 stage. However. by V15 and more advanced stages, maximum yield was obtained at SLNwp of 1.5 g N m^{-2} as suggested by Muchow and Sinclair (1994). Also above this threshold value of SLNwp, all plants had whole plant N concentrations above the critical level suggested by the Greenwood et al., 1990 equation. LN_{la} was superior to SLN_{WD} as a predictor of N status at early vegetative growth stages. This suggests that LN_{la}. a population dependent diagnostic. more accurately reflects ecosystem N supplyldemand than individual whole plant SLN.

Figure 5. Grain yield and leaf area index at VT stage as a function of fertilizer N rate for the four hybrids in experiment 1.

Figure 4. Temporal change in whole plant specific leaf N (SLNwp) and leaf N per unit land area (LNla) as a function of N rate. (Growth stages are indicated on the **s**axis).

N Rate			MAIZE GROWTH STAGE							
(kg N/ha)	V8	V15		R4						
	$-SLW_{dl}$ (g m-2 leaf)---									
0	48.00	50.34	54.07	66.32	54.68					
80	48.78	52.21	55.42	66.09	55.63					
160	50.10	51.68	53.51	66.56	55.56					
240	50.57	51.70	56.41	69.35	57.00					
	49.36	51.48	54.85	67.08						

Table 1 Change in specific leaf weight with plant age and N rate (average for site/hybrid).

Figure 6. Relationship between SLNwp and relative grain yield at four growth stages. Filled symbols are observations on or above the "Greenwood" curve.

Figure 7. Relationship between LNla and relative grain yield at four growth stages.

Experiment 2:(Population x N management)

Table 2 shows the results of the impact of population density and N management studied in the Lincoln. NE experiment. Population significantly increased LA1 at all measured growth stages. Final LAI (V12-VT stage) at 30M pl acre⁻¹ was similar to that observed in experiment 1. Grain yields were high and only the 37M population under high N dosage (M2) exhibited a significant increase in yield. We have deterrnined that the loss of yield at the highest population in the M2 treatment was due to the high respiratory cost of excessive leaf biomass. In all treatments. SLNwp exceeded 1.5 g N $m²$ leaf suggesting that N was not a limiting factor in this study. It is interesting to note, however. that SLNwp exhibited the same decline with plant age as was observed at Mead experiment 1. Although N silfficiency at Lincoln precluded determination of population driven threshold values of SLNwp or LNla, it is evident at SLNwp near 1.5 g N m⁻² **(44M** pl a-'. Vl2 and VT). LNla values were far in excess of those observed in experiment 1 suggesting that LNla is a more population dependent diagnostic of N demand.

In conclusion, although differences in yield and leaf development were observed among hybrids, there was no direct effect of hybrid on differences in leaf N. Both SLNwp and LNla were good measures of corn N status and LNla was superior to SLNwp as a predictor of N status at early vegetative stages. Grain yield was optimized at a SLNwp >1.5 g N m-2 leaf at mid to late vegetative stages. This threshold SLNup also determined the "Greenwood" curve implying a consistent and conserved relationship between biomass yield and N content of Cq plants. We have found that non-destructive prediction of maize N status (e.g. SPAD) is improved when LAI is factored in. Remote sensing of canopy N status will most likely be improved when sensing devices that adequately measure canopy **LA1** are coupled with algorithms that relate canopy reflectance to SLNwp

	Popul.		Leaf Area Index $m2$ leaf m ⁻² land area		Specific Leaf N $g \text{ N m}^2$ leaf		Leaf N/land area $g N m-2$ land area
Stage	$Mp a^{-1}$	N management level					
		$M1$	$\underline{M2}$	M1	M ₂	M1	M2
V6	30	0.57	0.73	2.14	2.24	1.21	1.64
	37	0.95	1.20	2.16	2.19	2.05	2.63
	44	1.26	1.44	1.99	2.04	2.49	2.94
		Pop**		NS		Pop**, Man*	
V12	30	4.96	4.61	1.72	2.05	8.53	9.40
	37	5.57	5.74	1.66	1.94	9.24	10.60
	44	6.54	6.32	1.59	1.66	10.40	10.54
		Pop**		Man**		Man *	
VT	30	4.84	4.42	1.96	2.33	9.52	10.24
	37	5.67	5.97	2.09	2.05	11.84	12.18
	44	6.60	6.68	1.76	.1.82	11.59	12.14
		Pop**		Pop x Man *		Pop x Man *	
Yield	30	225	229				
(bu/a)	37	233	248				
	44	231	232	Pop x Man**			

Table 2. Yield. LAI, SLNwp and LNla as a function of plant population and N management level. Experiment 2, Lincoln. NE.

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