Nitrogen status in maize grown at different row spacings and nitrogen availability

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Barbieri, P. A., Echeverría, H. E., Sainz Rozas, H. R. and Andrade, F. H. 2013. **Nitrogen status in maize grown at different row spacings and nitrogen availability**. Can. J. Plant Sci. **93**: 1049–1058. Improving nitrogen use efficiency (NUE) is imperative to sustainable agriculture. To attain this goal in maize crops (*Zea mays* L.) there are nitrogen (N) diagnosis methods that enable determination of a crop's nutritional status by analysis of plant parts. Maize planted in narrow rows (NR) can have increased dry matter (DM), grain yield and accumulated N. However, no reports have been found on the effect of NR of N in plant diagnosis methods. An experiment was performed over 3 yr to evaluate NR and N fertilizer rates on the N dilution curve, N concentration in grain and chlorophyll content in maize. Treatments consisted of a factorial combination of row width (70, 52 and 35 cm) and N rate (0 to 180 kg N ha^{-1}). The N dilution curves adjusted for fertilized or control treatments were similar among row spacing. Nitrogen concentration in grain was correlated with relative yield (RY), and similar critical values for N response were similar between row spacings. Leaf chlorophyll content increased with N and NR; however, green index (GI) and N sufficiency index (NSI) values were not different between row spacing when correlated to RY. These results indicate that response thresholds to N fertilization determined on plant tissue for NR treatments were similar among row spacings. Thus, there is no need to adjust the response thresholds to N application based on row spacing, as NR did not cause any changes in physiological efficiency (PE) due to the determined proportional increases, both in accumulated N in DM and grain yield.

Key words: Maize, row spacing, nitrogen status

Barbieri, P. A., Echeverría, H. E., Sainz Rozas, H. R. et Andrade, F. H. 2013. **Statut de l'azote chez le maïs cultivé à différents écartements entre lignes et disponibilité de l'azote**. Can. J. Plant Sci. **93**: 1049–1058. Améliorer l'efficacité utilisation de l'azote (NUE) est impératif pour une agriculture durable. Pour atteindre cet objectif dans les cultures de maïs (*Zea mays* L.), méthodes de diagnostic sont utilisés pour permettre déterminer l'état nutritionnel de N d'une culture par l'analyse des parties de la plante. Le maïs planté en rangs serrés (NR) peuvent avoir une plus matière sèche (MS), rendement en grains et azote (N) accumulé. Toutefois, aucun rapport sont disponibles à l'égard de l'effet de la NR sur le N pour les méthodes de diagnostic des cultures Une expérience a été réalisée pour trois ans afin d'évaluer l'effet de NR et des taux de N sur la courbe de dilution du N, la teneur en azote dans les grains et la teneur en chlorophylle dans le maïs. Les traitements consistaient en une combinaison factorielle la distance entre les rangées (70, 52 et 35 cm) et le taux de N (0 à 180 kg N ha^{-1}). Des courbes de dilution ajustés pour des traitements fertilisés et le contrôle étaient similaires entre les traitements d'espacement de rangée La concentration en N dans le grain a été corrélé avec le rendement relatif (RR), valeurs critiques de la réponse à N étaient similaires entre les distances entre les lignes. Le contenu en chlorophylle des feuilles augmenté avec la fertilisation azotée et la distance entre les lignes, mais vert l'indice (GI) et indice de suffisance N (INS) des valeurs n'étaient pas différentes entre l'écartement des rangs quand corrélée à RY. Ces résultats indiquent que le seuil de réponse à la fertilisation azotée déterminée dans les tissus végétaux pour les traitements NR étaient similaires pour les différents espacements. Il n'ya donc pas besoin d'ajuster le seuil de réponse à la demande de N sur la base de l'écartement des rangs, parce que le NR n'a pas causé de changements dans l'efficacité physiologique (PE) en tant que conséquence des augmentations proportionnelles déterminées, à la fois dans N accumulés dans le rendement DM et le grain.

Mots clés: Maïs, distance entre les lignes, statut d'azote

Correct nitrogen (N) nutrition diagnosis and monitoring is essential for maize production as the crop's N demands are high. Maize produced in temperate-humid areas requires different N rates depending on the applied management practices. Compared with conventional till systems, no-till (NT) systems require more N due to changes in the soil that can decrease N mineralization and nitrification while increasing N immobilization, denitrification, and/or leaching (Fox and Bandel 1986). To effectively increase nitrogen use efficiency (NUE) the

Abbreviations: **CET**, crop evapotranspiration; **DM**, dry matter; **GI**, green index; **NT**, no-till; **NR**, narrow rows; **NSI**, nitrogen sufficiency index; **NUE**, nitrogen use efficiency; **PE**, physiological efficiency; **RE**, recovery efficiency; **RY**, relative yield correct N availability diagnosis is required to avoid N application rates that exceed crop requirements. To evaluate N availability, there are different N diagnosis methods based on soil and plant analysis.

Plant tissue analysis is advantageous compared with soil analysis, requiring less time and effort to obtain a sample, and since plants represent an integration of factors related to soil N availability (Binford et al. 1992; Echeverría et al. 2000). Tissue analysis is based on the direct determination of nutrient concentration in plants or plant parts in key crop development stages. As a crop grows and accumulates dry matter (DM), a decline in total N concentration occurs, even without N limitations present (Greenwood et al. 1990). Nitrogen critical concentration is the N concentration that allows a crop to reach its maximum growth rate. The N critical concentration decreases as above-ground biomass increases; these two-plotted variables are referred to as critical nitrogen dilution curves. These curves were developed for crops such as tall fescue (*Festuca arundinacea* Schreb.) (Lemaire and Salette 1984), wheat (*Triticum aestivum* L.) (Justes et al. 1994), rice (*Oryza sativa* L.) (Sheehy et al. 1998), grain sorghum (*Sorghum bicolor*) (van Oosterom et al. 2001), and maize (Plenet and Lemaire 2000). An N nutrition index is generated from the ratio of actual N concentration to N critical concentration, which is obtained from the critical nitrogen dilution curves (Lemaire et al. 1989). N concentrations lower than the critical limit can cause various degrees of crop stress resulting in lower than maximum crop growth rates.

Nitrogen concentration in grain at physiological maturity is another N nutrition indicator used in maize. This methodology is helpful to determine whether there are crop N deficiencies or excesses (Pierre et al. 1977a, b; Lubert and Juste 1985; Cerrato and Blackmer 1990; Uhart and Andrade 1995), in order to correct the fertilization rates for the next crop cycle (Uhart and Echeverría 2002). Values mentioned in the above references set an approximate threshold at approximately 12.0 g kg^{-1}; above this value yield is not expected to change with an increase in N supply.

A common disadvantage of these methods is the effort required to obtain and process sample, and the time needed for sample analysis (Sainz Rozas and Echeverría 1998). Chlorophyll content in maize is highly correlated with leaf N concentration and therefore it can be used to evaluate maize nutritional content (Wolfe et al. 1988). The Minolta SPAD-502 portable leaf chlorophyll meter (Konica Minolta, Osaka, Japan) measures chlorophyll content indirectly and non-destructively, allowing crop nutritional condition to be provided simply by a reading indicated by the green index (GI). This chlorophyll meter has been successfully used to determine N status for many crops such as wheat, maize, rice, and potato (*Solanum tuberosum* L.) (Blackmer and Schepers 1995; Sainz Rozas and Echeverría 1998; Gandrup et al. 2004; Giletto et al. 2006). The relationship between SPAD readings and crop yield for the majority of the crops was poor at early crop developmental stages, but improved at later stages due to a greater expression of N deficiency as crop requirements increased. An N sufficiency index (NSI) has been proposed to minimize differences among hybrids, environments and other factors that affect chlorophyll content, in order to compare different N conditions. An NSI value between 0.92 and 0.98 is needed to achieve 95% of maximum yield in maize (Sainz Rozas and Echeverría 1998).

Narrow row spacing (NR) increases maize crop yield (Fulton 1970; Hunter et al. 1970; Stivers et al. 1971; Ottman and Welch 1989; Porter et al. 1997; Barbieri et al. 2000; Andrade et al. 2002) due to a greater intercepted radiation at flowering (Ottman and Welch 1989; Barbieri et al. 2000; Andrade et al. 2002). Early in the maize-growing season (sowing–vegetative six leaf, V6) it was determined that low N rates in NR increased N recovery efficiency (RE) of available N, thus improving NUE (Barbieri et al. 2008). Furthermore, NR accumulated N in similar proportions, and had greater grain yields and stover DM than wider row spacings, thus not affecting physiological efficiency (PE) (Barbieri et al. 2008). This lack of change in PE indicates that the same dilution curve could be adjusted for different row spacing without requiring the threshold values of NSI and grain N concentration to be different between row spacing. Shapiro and Wortmann (2006) reported that reduced row spacing without increasing plant density resulted in more crop N uptake and grain yield. However, the prediction of optimal N fertilization rate cannot be improved if plant density and row spacing are the only factors considered. Ma et al. (2003) found that NR did not result in higher yield or NUE, even when plant density and N fertility varied. There is little knowledge available regarding the effects of row spacing on plant N diagnosis methods. Thus, the objective of this research was to determine maize N thresholds for different row spacing using plant N diagnosis.

MATERIALS AND METHODS

The experiments were conducted over 3 yr under NT at the Instituto Nacional de Tecnología Agropecuaria (INTA) Research Station, Balcarce (lat. 37°45′S, long. 58°18′W; 130 m above sea level, 870 mm mean annual rainfall, 13.7°C mean annual temperature), Buenos Aires, Argentina. This area is characterized with a low average temperature during the growing season and a frost free period of approximately 150 d. Andrade and Gardiol (1995) provide more details regarding the climatic data for this site.

The soil consisted of fine mixed Typic Argiudoll and fine thermic Petrocalcic Paleudoll. Other soil characteristics determined at planting are presented in Table 1.

The experimental design was a split-plot in randomized complete block with three replications. In 1996–1997 the main plot measured effects of row spacing (conventional rows 70 cm and narrow rows 35 cm between rows, at 7.65 plants m^{-2}) and the sub-plot measured effects of N rate (0 and 140 kg of N ha^{-1}). In 1999–2000 and 2000–2001,

Table 1. Soil characteristics at maize planting at the Instituto Nacional de Tecnología Agropecuaria (INTA) Research Station, Balcarce

Year	P^z (0–20 cm) (mg kg^{-1})	NO_3^--N^y (0–60 cm) (kg ha^{-1})	pH	OC^x (0–20 cm) (g ha^{-1})
1996–1997	21.4	32	5.9	32.0
1999–2000	17.2	54^y	6.0	32.1
2000–2001	36.9	31^y	6.1	32.1

^{z}P, available P (Bray and Kurtz 1945).
^{y}Values of NO_3^--N are average across row spacings.
^{x}OC, organic carbon.

the main plot measured effects of N rate (0, 90 and 180 kg N ha^{-1}) and the sub-plot measured effects of row spacing (conventional rows 70 cm and narrow rows 52 and 35 cm between rows, at 7.9 plants m^{-2}). Additions to N rates and row spacing treatments were applied to estimate the row spacing effects more accurately.

For all row spacings, the experimental units (subplots) were 14 m long and seven rows wide (two border rows on each side). The fertilizer was urea (46% N) broadcast without incorporation at the V6 growth stage. During each growing season, plots were fertilized at planting with 20 kg P ha^{-1} and irrigated as needed. Weeds and insects were adequately controlled, to avoid crop growth limitations. Barbieri et al. (2008) provide more details regarding crop production at this site (hybrids, plant population, weeds and insects control, etc.).

Crop evapotranspiration (CET) was determined as the product between potential evapotranspiration (ET_0) and crop coefficient (K_c). The ET_0 was calculated according to Pennman (1948). Della Maggiora et al. (2000) report the K_c (CET/ET_0) for the area. Ten maize plants were collected from three inner rows to determine above-bground DM accumulation at vegetative six leaf V6 (Ritchie and Hanway 1982), pre-flowering (vegetative 12 leaf V12, 15 d before flowering), post-flowering (reproductive milk stage R3, 15 d after flowering), and R6 (physiological maturity) growth stages. Plants were cut at ground level, oven dried, weighed, and milled to pass through a 1-mm mesh. Reduced-N was determined by Method A (without salicylic acid modification) as reported by Nelson and Sommers (1973). Total reduced-N accumulated was calculated as N concentration (dry weight basis) multiplied by DM.

Leaf chlorophyll content was evaluated at the V6, pre-flowering (V12) and post-flowering (R3) growth stages using a Minolta SPAD 502 chlorophyll meter. Meter readings were taken on the uppermost fully expanded leaf with a visible collar during vegetative growth, and from the ear leaf during reproductive growth (20 plants per treatment) as suggested by Blackmer and Schepers (1995). The NSI was estimated using experimental unit chlorophyll readings for each row spacing treatment by a ratio of row spacing treatment to the highest N supply treatment (140 kg ha^{-1} in 1996/1997 and 180 kg ha^{-1} in 1999/2000 and 2000/2001). Relative yield values were correlated with chlorophyll reading and NSI.

Crop nutritional status was determined through the dilution curve proposed by Plénet and Lemaire (2000), where the N concentration in DM needed to attain maximum crop growth rate was expressed by the equation $N = 34.0DM^{-0.37}$. Dilution curves to describe N concentration in DM and shoot biomass were generated using an exponential model for fertilized and control treatments. This model was linearized by applying the logarithm of N concentration and accumulated DM. Coincidence and parallelism tests ($P > 0.05$) were applied to compare slopes and intercepts using SAS software (SAS Institute, Inc. 1985).

Relative yield (RY) was determined by dividing the yield from each row spacing treatment by the average yield from treatments with the highest N rate. Relative yields of NR (35 and 52 cm) were pooled because no significant differences were found in grain yield between treatments. A linear-plateau regression model was used to describe the relationship between RY and N concentration in grain for all growing seasons. Linear-plateau and lineal regression models were fitted using the NLIN and REG procedures of SAS software, respectively (SAS Institute, Inc. 1985).

The critical concentration of N was determined where grain N concentration intersected with the linear and plateau segments. Data were analyzed using a general linear model that included treatment and block effects. Treatment effects were evaluated for each year by ANOVA using SAS software (SAS Institute, Inc. 1985). Following an *F*-test in ANOVA, multiple comparisons of means were conducted with a Tukey's test.

RESULTS AND DISCUSSION

During each growing season, water availability did not limit maize yield, as rainfall and irrigation exceeded maize crop evapotranspiration during the critical period for kernel set (January) (Table 2). Analyses of grain yield, and NUE, PE and RE for DM and grain were published in Barbieri et al. (2008).

Above-ground Dry Matter and Nitrogen Accumulation

Dry matter during the growing season ranged from 148 to 22.832 kg DM ha^{-1}, depending on row spacing, N application rate, phenological stage, and year (Table 3). Dry matter increased significantly with N fertilization in nine of eleven phenological stages during the 3 yr of evaluation (Table 3).

In general, DM accumulation was not different between row spacing (Table 3). NR increased DM accumulation only during pre-flowering and physiological maturity in 1999–1900, and pre-flowering in 2000–2001 (Table 3). A significant interaction between N rate and row spacing for DM was found only in 1999–2000 at physiological maturity due to a greater relative increment in DM obtained for narrow rows treatments

Table 2. Rainfall, irrigation and maize crop evapotranspiration (CET) during three maize growing seasons at Balcarce Argentina

	1996–1997			1999–2000			2000–2001		
Month (stage)	Rainfall	Irrigation	CET	Rainfall	Irrigation	CET	Rainfall	Irrigation	CET
	(mm)								
Oct. (planting)	176		12	66		13	97		12
Nov.	89		76	50		74	35		71
Dec.	116		133	66	106	144	83	66	151
Jan. (silking)	100	139	159	122	156	168	119	116	175
Feb.	119		103	225		97	119	74	105
Mar. (black layer)	153		43	164		30	106		36
Total	892		527	955		526	815		550

without N fertilization (Table 3). Cox and Cherney (2002) reported greater DM accumulation at physiological maturity with NR (38 vs. 76 cm) in only 2 of 9 site-year comparisons. Increments in DM accumulation from NR were also reported by other authors (Bullock et al. 1988; Cox et al. 1998, 2006).

Nitrogen accumulation varied from 5 to 179 kg DM ha^{-1} depending on row spacing, N application rate, phenological stage, and year (Table 4). Nitrogen fertilization increased crop N accumulation in most phenological stages (Table 4). Narrow rows significantly increased N accumulation in above-ground DM in 6 of 12 phenological stages (Table 4). A significant interaction between row spacing and N rate was found at preflowering in 1996–1997 and V6 in 1999–2000 due to greater relative increments for N accumulation in NR treatments without N fertilization (Table 4). Narrow rows treatments displayed a greater N accumulation

Table 3. Above-ground dry matter accumulation of irrigated maize crop under no till during the 1996/1997, 1999/2000 and 2000/2001 growing seasons based on row spacing and N fertilizer rate

Treatments		Phenological stage											
		1996–1997				1999–2000				2000–2001			
RS^z (cm)	N rate (kg ha^{-1})	V6^z	Pref^z	Posf^z	PM^z	V6	Pref	Posf	PM	V6	Pref	Posf	PM
		(kg ha^{-1})											
70	180	–	–	–	–	775	5615	12 513	19 348*a*	435	5173	12 914	16 646
52	180	–	–	–	–	762	6156	12 907	18 861*a*	459	5915	12 671	17 222
35	180	–	–	–	–	737	5803	12 834	19 858*a*	452	5372	13 252	16 475
70	140	–	4176	13 773	20 671	–	–	–	–	–	–	–	–
35	140	–	4425	13 866	22 832	–	–	–	–	–	–	–	–
70	90	–	–	–	–	687	5277	12 896	17 152*a*	408	5519	11 373	15 257
52	90	–	–	–	–	681	5953	13 005	17 817*a*	420	5773	12 628	15 827
35	90	–	–	–	–	776	5813	12 574	17 305*a*	403	5422	12 384	15 879
70	0	148	4025	12 270	15 593	483	3838	9 020	12 266*b*	278	4471	10 374	11 058
52	0	–	–	–	–	550	4927	10 679	15 478*a*	385	4558	9 607	11 742
35	0	319	3923	12 085	17 333	563	4200	9 681	13 761*a*	391	4646	10 878	12 140
Avg. N rate	180	–	–	–	–	752*a*	5858*a*	12 751*a*	19 356	449*a*	5487*a*	12 946*a*	16 780*a*
	140	–	4300*a*	13 820*a*	21 751*a*	–	–	–	–	–	–	–	–
	90	–	–	–	–	715*a*	5681*a*	12 825*a*	17 425	410*a*	5571*a*	12 128*a*	15 654*b*
	0	–	3974*a*	12 177*b*	16 637*b*	532*b*	4522*b*	96 83*b*	13 499	352*a*	4558*b*	10 286*b*	11 647*c*
Avg. RS	70	148*a*	4100*a*	13 021*a*	18 640*a*	648*a*	4910*b*	11 476*a*	16 255	374*a*	5054*b*	11 553*a*	14 321*a*
	52	–	–	–	–	665*a*	5678*a*	12 387*a*	17 493	421*a*	5415*a*	11 635*a*	14 929*a*
	35	319*a*	4174*a*	12 975*a*	20 082*a*	692*a*	5272*ab*	11 696*a*	16 975	415*a*	5147*ab*	12 172*a*	14 832*a*
ANOVA													
N		–	NS	***	*	*	**	**	*	NS	**	*	*
RS		NS	NS	NS	NS	NS	**	NS	**	NS	**	NS	NS
N × RS		–	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
CV (%)		35.4	10.7	8.6	1.4	10.3	9.2	5.6	4.1	11.9	5.3	5.6	5.3

^{z}RS, row spacing; V6, Pref, Posf and PM refer to the six leaves, pre-flowering (15 d before), post-flowering (15 d after) and physiological maturity, phenological stages, respectively.
*, **, *** Significant at the 1, 5 and 10% probability levels, respectively.
a–c Values within each column for a given main effect or interaction followed by the same letter are not significantly different from each other based on the Tukey test.

Table 4. Nitrogen accumulation of irrigated maize crop under no till during the 1996/1997, 1999/2000 and 2000/2001 growing seasons based on row spacing and N fertilizer rate

Treatments		Phenological stage													
		1996–1997				1999–2000				2000–2001					
RS[z] (cm)	N rate (kg ha^{-1})	V6[z]	Pref[z]	Posf[z]	PM[z]	V6	Pref	Posf	PM	V6	Pref	Posf	PM
		(kg ha^{-1})											
70	180	–	–	–	–	25*a*	91	139	174	14	81	117	122
52	180	–	–	–	–	25*a*	101	134	164	16	92	123	133
35	180	–	–	–	–	24*a*	99	145	172	15	86	116	124
70	140	–	92*a*	199	170	–	–	–	–	–	–	–	–
35	140	–	88*a*	167	179	–	–	–	–	–	–	–	–
70	90	–	–	–	–	21*a*	72	118	130	14	72	90	106
52	90	–	–	–	–	20*a*	71	106	129	14	89	93	109
35	90	–	–	–	–	24*a*	81	109	129	13	78	101	110
70	0	5	48*b*	87	88	12*b*	43	57	79	8	51	67	67
52	0	–	–	–	–	14*a*	55	63	99	12	62	73	76
35	0	11	55*a*	108	113	14*a*	51	66	89	12	52	77	76
Avg. N rate	180	–	–	–	–	24	97*a*	140*a*	170*a*	15*a*	86*a*	118*a*	126*a*
	140	–	90	183*a*	175*a*	–	–	–	–	–	–	–	–
	90	–	–	–	–	22	74*b*	111*b*	129*b*	14*a*	79*b*	95*b*	108*b*
	0	–	52	97*b*	101*b*	14	50*c*	62*c*	88*c*	11*a*	55*c*	72*c*	73*c*
Avg. RS	70	5*b*	70	143*a*	129*b*	19	69*b*	105*a*	128*a*	12*b*	68*b*	91*a*	98*b*
	52	–	–	–	–	20	75*ab*	106*a*	134*a*	14*a*	81*a*	95*a*	106*a*
	35	11*a*	72	138*a*	146*a*	21	77*a*	107*a*	130*a*	14*a*	72*b*	98*a*	103*a*
						ANOVA							
N		–	*	*	*	*	*	*	*	NS	**	*	*
RS		**	NS	NS	***	NS	***	NS	NS	**	**	NS	***
N × RS		–	***	NS	NS	***	NS	NS	NS	NS	NS	NS	NS
CV (%)		12.7	5.5	20.4	5.1	7.6	9.8	10.5	9.0	11.1	7.3	10.9	6.5

[z] RS, row spacing; V6, Pref, Posf and PM refer to the six leaves, pre-flowering (15 d before), post-flowering (15 d after) and physiological maturity, phenological stages, respectively.

*, **, *** Significant at the 1, 5 and 10% probability levels, respectively.

a–c Values within each column for a given main effect or interaction followed by the same letter are not significantly different from each other based on the Tukey test.

predominantly at V6 and pre-flowering phenological stages (Table 4). These results indicate a greater N uptake capacity by NR compared with conventional row (CR) and, thus, an incremental increase in RE of available N (Barbieri et al. 2008). Relative increments (%) in N accumulation in response to NR (averaged across all growing seasons and N rate) were 26, 8, 1, and 8% for V6, pre-flowering, post-flowering and physiological maturity, respectively. Cox and Cherney (2002) reported similar plant N concentrations at V6, ear-leaf N concentrations at silking, and whole-plant N concentrations at harvest for different row spacing (38 cm, 76 cm and 38 cm at high density). Increases in N accumulation from NR treatments were reported by Rosolem et al. (1993) and Cox and Cherney (2001).

Dilution Curve

The critical N dilution curves by Plenet and Lemaire (2000) discriminated between the limiting and non-limiting N conditions (Fig. 1a and c). The points with N concentrations below the curve are N stress situations and points above the curve are excess N situations. Essentially there was indication of some N stress since treatments fertilized with the maximum N rate (140 and 180 kg N ha^{-1}, years 1, and 2 and 3, respectively) had N concentrations below the N critical curve (Fig. 1a).

Linearized data using the logarithm of N concentration versus accumulated DM presented curves that were significantly different (N critical curve vs. fertilized and control treatments) (Fig. 1b and d). Yields obtained from fertilized treatments were high (11.15 Mg ha^{-1} average for 3 yr). Yet, the generated curves indicated that the N fertilizer rate was insufficient, or the dilution curve proposed by Plénet and Lemaire (2000) was too high, implying the need to calibrate a curve for local conditions. These results differ from those reported by Ziadi et al. (2008), who determined that the Plénet and Lemaire (2000) curve was valid for Eastern Canada conditions, as did Herrmann and Taube (2004) for Germany.

Nitrogen concentrations for control treatments were below the N critical curve showing a greater N stress compared with fertilizer treatment (Fig. 1c). Nitrogen dilution curves for fertilized and control treatments (Fig. 1b and d) were linearized by applying the logarithm of N concentration and accumulated DM; generating slopes

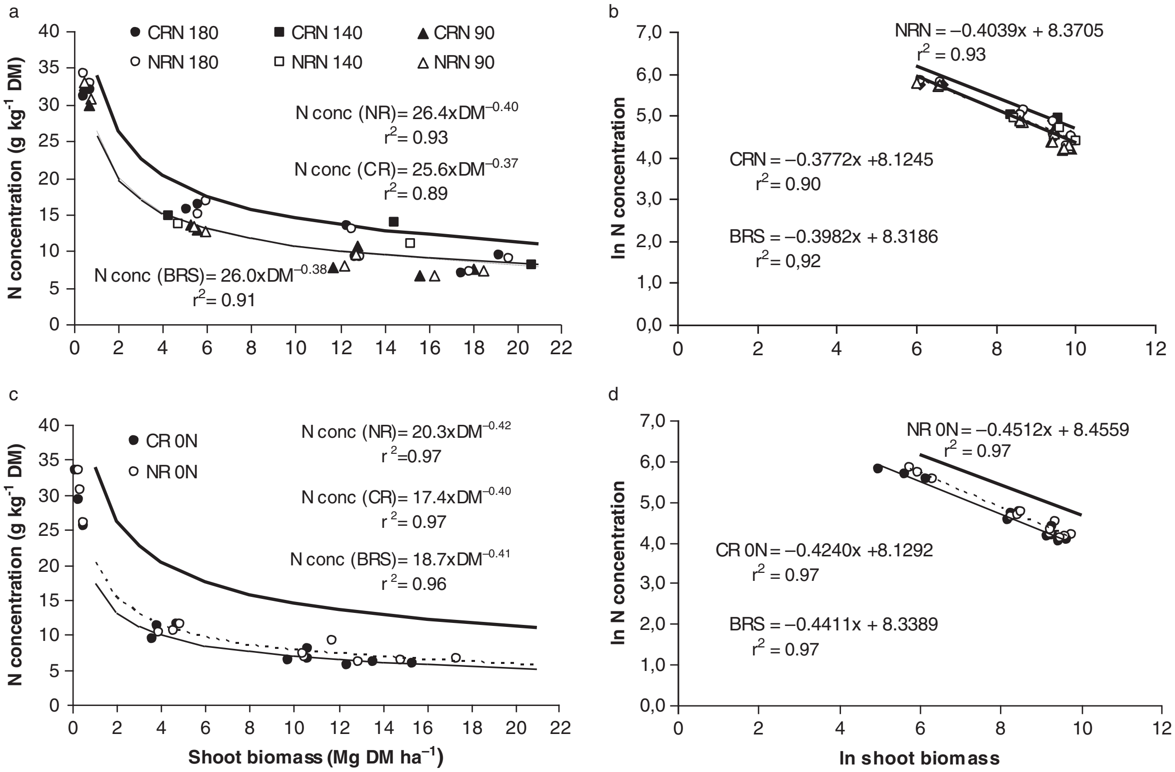

Fig. 1. Nitrogen concentration in aerial biomass of irrigated maize under no till (a and c) and logarithm LN of nitrogen concentration in aerial biomass (b and d), based on row spacing and N supply for three growing seasons. CR 0N, conventional row spacing without N fertilizer; CRN, conventional row spacing with N fertilizer; NR 0N, narrow row spacing without N fertilizer; NRN, narrow row spacing with N fertilizer. Broken line represents adjustments for treatments with conventional and narrowed row spacing, respectively. BRS, both row spacings combined. Solid thicker line represents the reference curve as proposed by Plenet and Lemaire (2000) (N conc = 34.0 DM–0.37).

and ordinates at the origin did not differ between row spacing ($P > 0.05$). Therefore, a single model was developed for both row spacings in the fertilized and control treatments: $N = 26.0x^{-0.38}$ $r^2 = 0.91$ and $N = 18.7x^{-0.41}$ $r^2 = 0.96$ (with x being shoot biomass in Mg DM ha^{-1}), respectively. A significant ($P < 0.01$) linear relationship ($r^2 = 0.80$) was determined between relative N accumulation and DM increments (%) in response to NR spacing (Fig. 2). Accumulated N and DM increased in similar proportions; therefore, no changes in PE were determined (Barbieri et al. 2008). Narrow rows without N fertilization treatments had the greatest relative increments in N concentration and DM (Fig. 2) indicating that when NR treatments have a higher N accumulation and greater NUE compared with CR (Barbieri et al. 2008), it may not be necessary to adjust curves based on row spacing.

Nitrogen Concentration in Grain

Nitrogen concentration in grain was correlated with RY for both NR and CR row spacings (CR, $RY = 13.1NG - 48.3$ if $NG < 11.0$, $r^2 = 0.63$, and NR, $RY = 15.2NG - 67.4$ if $NG < 10.7$, $r^2 = 0.53$). The thresholds determined in this experiment are similar to those reported by Pierre et al. (1977b) and slightly lower than the 12 g kg^{-1} that was established for Balcarce by Uhart and Andrade (1995). Despite the higher grain yields observed for NR treatments, slope and ordinate at origin, as well as

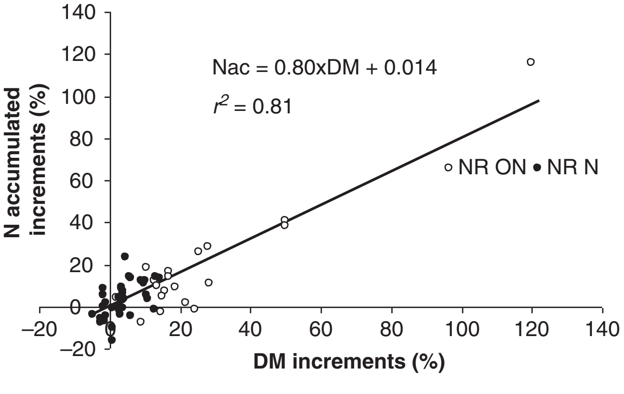

Fig. 2. Relationship between relative N accumulation increments (%) and DM increments (%) in response to reductions in row spacing. NR 0N, narrow row spacing without N fertilizer; NRN, narrow row spacing with N fertilizer.

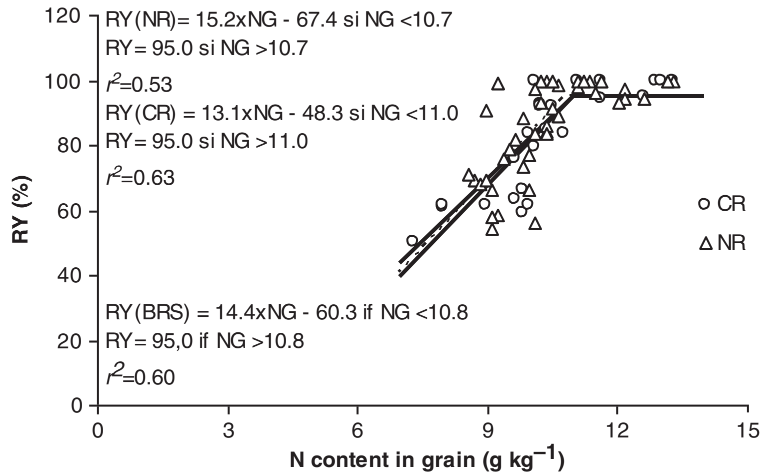

Fig. 3. Relationship between relative grain yield (RY) and N content in grain (NG) in irrigated maize under NT, based on row spacing and N supply for three growing seasons. CR, conventional spacing of 70 cm; NR, narrowed spacing of 35 or 52 cm; BRS, both row spacings combined.

critical concentration to attain 95% of maximum yield, were not different ($P > 0.05$) between row spacings. Therefore, a single model was developed for both row spacings ($RY = 14.4NG - 60.3$ if $NG < 10.8$, $r^2 = 0.60$) (Fig. 3), indicating that NR treatments increased grain yield and N uptake similarly, mainly at low N

Table 5. Leaf green index of irrigated maize crop under no till during the 1996/1997, 1999/2000 and 2000/2001 growing seasons based on row spacing and N fertilizer rate

Treatments		Phenological stage								
		1996–1997			1999–2000			2000–2001		
RS[z] (cm)	N rate (kg ha^{-1})	V6[z]	Pref[z]	Posf[z]	V6	Pref	Posf	V6	Pref	Posf
		Unidades SPAD								
70	180	–	–	–	48.5	49.9*a*	52.1*a*	48.6	51.5	48.1
52	180	–	–	–	48.5	50.9*a*	54.3*a*	50.0	52.1	49.2
35	180	–	–	–	49.1	51.3*a*	53.2*a*	50.5	52.8	46.6
70	140	–	52.7	54.4	–	–	–	–	–	–
35	140	–	52.2	55.6	–	–	–	–	–	–
70	90	–	–	–	47.4	48.0*a*	47.6*a*	48.1	48.1	40.2
52	90	–	–	–	47.5	47.3*a*	48.3*a*	48.5	49.2	41.9
35	90	–	–	–	49.0	47.5*a*	47.8*a*	49.9	49.3	40.7
70	0	43.7	38.3	37.9	41.6	35.8*b*	35.5*b*	45.6	39.8	30.3
52	0	–	–	–	43.4	40.7*a*	40.4*a*	46.7	40.2	32.5
35	0	47.1	41.2	39.3	43.8	38.0*ab*	37.9*ab*	47.3	39.9	31.4
Avg. N rate	180	–	–	–	48.6*a*	50.8	53.2	49.7*a*	52.1*a*	48.0*a*
	140	–	52.5*a*	55.0*a*	–	–	–	–	–	–
	90	–	–	–	48.0*a*	45.6	47.9	48.8*a*	48.9*b*	40.9*b*
	0	–	39.7*b*	38.6*b*	42.9*b*	37.5	36.8	46.6*b*	40.0*c*	31.4*c*
Avg. RS	70	43.7*b*	45.5*a*	46.2*a*	45.8*b*	44.5	45.1	47.4*b*	46.5*a*	39.5*b*
	52	–	–	–	46.4*b*	46.7	48.4	48.4*ab*	47.2*a*	41.2*a*
	35	47.1*a*	46.7*a*	47.5*a*	47.3*a*	45.6	46.3	49.2*a*	47.3*a*	39.5*b*
					ANOVA					
N		–	*	*	*	*	*	***	*	*
D		**	NS	NS	*	**	*	**	NS	***
N × D		–	NS	NS	NS	**	**	NS	NS	NS
CV (%)		1.4	1.5	2.8	1.7	2.5	1.8	2.7	2.3	4.0

[z] RS, row spacing; V6, Pref and Posf refer to the six leaves, pre-flowering (15 d before) and post-flowering (15 d after) phenological stages, respectively.

*, **, *** Significant at the 1, 5 and 10% probability levels, respectively.

a–c Values within each column for a given main effect or interaction followed by the same letter are not significantly different from each other based on the Tukey test.

availability (Barbieri et al. 2008). These results suggest that different models or thresholds for N fertilization are not needed for different row spacings.

Chlorophyll Content

The GI varied from 30.3 to 55.6 units SPAD depending on row spacing, N application rate, phenological stage, and year (Table 5). In all growing seasons, N application increased GI significantly during all phenological stages (Table 3). Average GI (across growing seasons) increased with N rate from 38.7 to 49.3 for control and fertilized treatments, respectively. In agreement with previous studies (Piekielek and Fox 1992; Schepers et al. 1992; Dwyer et al. 1995), the application of the GI as a method to determine N status in maize was adequate and provided rapid diagnostic methods. Narrow rows increased GI significantly in six of nine phenological stages, with a higher increase of GI in treatments without N fertilizer applications (Table 5), due to a greater N accumulation by NR compared with CR (Table 4). In the 1996–1997 growing season, GI differences between row spacings for control treatments were 3.4, 2.9 and 1.4 at the V6, pre-flowering and post-flowering stages, respectively (Table 5). In the 1999–2000 growing season, GI differences were 1.8, 4.9, and 4.9 for 52-cm row spacing, and 2.2, 2.2, and 2.4 for 35-cm row spacing at the V6, pre-flowering and post-flowering stages, respectively (Table 5). In the 2000–2001 growing season, the GI differences were 1.1, 0.4, and 2.2 for 52-cm row spacing and 1.7, 0.1, and 1.1 for 35-cm row spacing at the V6, pre-flowering and post-flowering stages, respectively (Table 5). The greater GI determined in NR compared with CR demonstrates that NR generate a better N nutritional condition for maize under NT (Table 5) resulting in higher N uptake by the crop (Barbieri et al. 2008). The average GI increased (average across years and rates) for NR relative to CR by 4.5, 2.5 and 2.2% at the V6, pre-flowering and post-flowering stages, respectively.

Green index values were correlated with RY ($P<0.05$) (Table 6). In agreement with others authors (Blackmer and Schepers 1995; Waskom et al. 1996; Bullock and Anderson 1998), the correlation coefficients were low at early developmental stages (V6 from 0.35 to 0.57), and increased as the growing season progressed (from 0.68 to 0.90) (Table 6). Therefore GI readings were not useful for predicting N fertilizer requirements during early stages; similar results were provided by Blackmer and

Table 6. Relationship between green index at different phenological stages and maize relative grain yield (RY) under NT based on row spacing and N fertilizer rate for three growing seasons[z]

Phenological stage	Regression equation	r^2	GI units to attain 95% of $Y_{\max}$
	Conventional row		
V6	RY = 4.1x–108.3	0.57	49.5
Preflowering	RY = 2.8x–44.4	0.90	49.7
Postflowering	RY = 2.0x–2.7	0.76	48.8
	Narrow row		
V6	RY = 3.1x–61.0	0.35	50.3
Preflowering	RY = 2.4x–23.7	0.80	49.5
Postflowering	RY = 1.6x + 18.1	0.68	48.1
	Both row spacings		
V6	RY = 3.4x–77.9	0.44	50.8
Preflowering	RY = 2.6x–33.3	0.84	49.2
Postflowering	RY = 1.7x + 9.3	0.71	50.3

[z] x, green index reading; $Y_{\max}$, maximum yield.

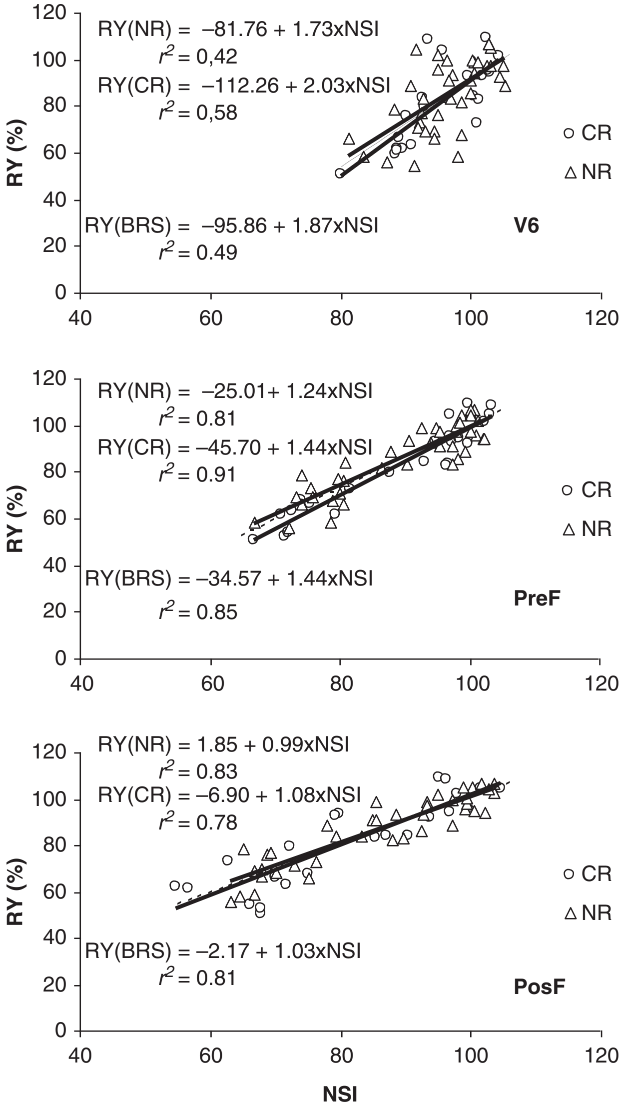

Fig. 4. Relationship between relative grain yield (RY) and N sufficiency index (NSI) in irrigated maize crop under NT, based on row spacing and N supply for three growing seasons. V6, Pref and Posf = six leaves, preflowering and postflowering phenological stages, respectively. CR, conventional row spacing; NR, narrowed row spacing; BRS, both row spacings combined.

Shepers (1995), and Sainz Rozas and Echeverria (1998). Green index units used to obtain 95% of maximum yield were not significantly different between row spacing; therefore, a single model was applied for both NR and CR row spacing (Table 6).

A significant positive relationship ($P < 0.05$) was determined between RY and NSI at V6, pre-flowering and post-flowering for CR and NR spacing (Fig. 4). The slopes and ordinates at the origin were not significantly different ($P > 0.05$) between row spacing; therefore, NSI values to achieve 95% of RY were similar for both row spacings. Similar to determining the RY and GI relationship (Table 6), correlation coefficients observed for RY and NSI relationships were low at V6, but higher pre- and post-flowering. These results agree with other studies (Blackmer and Shepers 1995; Sainz Rozas and Echeverría 1998; Zhang et al. 2008; Pagani et al. 2009). Considering the results, the chlorophyll meter Minolta SPAD 502 has limited potential as a diagnosis tool for N availability in early crop stages such as V6. A possible explanation for this limitation could be the low crop N requirements and air mean temperature at the site, since there is an inverse relationship that exists between average air temperature and chlorophyll concentration in maize leaves (Dwyer et al. 1991). Hence, small changes in average temperature at early stages may affect leaf chlorophyll concentration and, consequently, GI values independently from N availability (Sainz Rozas and Echeverría 1998). After V6, the average air temperature increased to the optimal range for chlorophyll synthesis and its concentration depended more on N availability. Correlations between RY and GI or NSI values were not affected by row spacing. However, higher leaf chlorophyll values occurred with NR treatments; this might be because NR proportionally increased grain and stover DM yields, and accumulated N (Barbieri et al. 2008). These results suggest that the same response thresholds for N fertilization could be applied for maize crops grown under different row spacing. Shapiro and Wortmann (2006) reported similar results for maize in the Midwest, where they concluded that prediction of optimal N rates could not be improved when only considering plant density and row spacing.

CONCLUSION

Results obtained indicate that N status in maize determined by plant analysis (dilution curve, GI, NSI and grain N concentration) were not affected by row spacing, presenting no advantage in developing new thresholds for N fertilization. The lack of NR influence on PE was associated with proportional increases in accumulated N and DM. Our study suggests that row spacing did not affect N fertilization thresholds. However, future studies should elucidate whether row spacing influences other N diagnostic methods; for example those methods based on spectral vegetation indices (i.e., Normalized Difference Vegetation Index).

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