Inbred maize response to cover crops and fertilizer-nitrogen[1,2]

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ABSTRACT

Maize (*Zea mays* L.) inbred seed production fields on the southern semiarid coast of Puerto Rico are usually fallow each year from May to September. Inbreds have lower seed yields than single-cross hybrids, yet producers tend to apply high fertilizer nitrogen (N) levels in efforts to increase yields. Inbred maize response to fertilizer-N was evaluated on the southern semiarid coast of Puerto Rico in a cover crop-maize cropping sequence in 2009, and in a fallow-maize sequence in 2010 in a Fluventic Haplustoll. In general, maize produced after a legume cover crop of velvetbean (*Mucuna prurience*) or cowpea (*Vigna unguiculata* 'Iron Clay') had better yields and agronomic traits than maize after the fallow treatment. In 2009, maximum seed yields of 2,726 kg/ha were obtained with fertilizer-N application in the range of 112 to 224 kg N/ha. In 2010, maximum seed yields of 1,447 kg/ha were obtained with fertilizer-N application in the range of 84 to 211 kg N/ha. Harvest index was 0.26 and 0.27 in 2009 and 2010 for all fertilizer-N treatments; higher than that for unfertilized maize. In general, agronomic traits were superior as a result of fertilizer-N application without consistent differences among fertilizer-N levels applied. The SPAD chlorophyll meter, leaf color index and leaf area index were suitable indicators of N status in the maize plants. Highest N use efficiencies were observed for the 112 kg N/ha and 84 kg N/ha fertilizer levels for 2009 and 2010, respectively, and decreased with increasing fertilizer-N applied. Fertilizer-N rates in soils, climatic systems, and maize inbreds similar to the ones tested should be between 84 and 112 kg N/ha. Greater amounts of fertilizer-N will result in decreased economic benefit and potential environmental contamination.

Key words: inbred maize, cover crops, fertilizer N, crop response

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RESUMEN

Respuesta del maíz a cultivos de cobertura y fertilización con nitrógeno

La producción de líneas puras de maíz (*Zea mays* L.) se realiza principalmente en la zona semiárida del sur de Puerto Rico, donde los predios están en barbecho sin cobertura vegetal de mayo a septiembre de cada año. Las líneas puras tienen menores rendimientos que los híbridos, pero los productores tienden a aplicar altos niveles de fertilizante nitrogenado (N) con la intención de aumentar los rendimientos. Se evaluó la respuesta de líneas puras de maíz a fertilizante-N en una secuencia de cobertura-maíz en 2009 y barbecho-maíz en 2010 en un Fluventic Haplustoll en la zona de los llanos costeros del sur de Puerto Rico. En general, el maíz producido luego de la cobertura de *Mucuna prurience* o *Vigna unguiculata* 'Iron Clay' mostró mayores rendimientos y mejores indicadores agronómicos que el maíz luego de barbecho. En el 2009, el rendimiento máximo en semilla de 2,726 kg/ha se logró con la aplicación de fertilizante-N en el rango de 112 a 224 kg N/ha, y en el 2010 el rendimiento máximo en semilla de 1,447 kg/ ha se logró con fertilizante-N en el rango de 84 a 211 kg N/ha. El índice de cosecha fue de 0.26 y 0.27 en 2009 y 2010 para los tratamientos fertilizados con N, y fue mayor que en maíz sin fertilizar. El medidor de clorofila en hoja, el índice de color en hoja y el índice de área foliar fueron buenos indicadores de suficiencia de N. La aplicación de fertilizante-N en suelos, clima y líneas puras de maíz similares a lo evaluado debe ser entre 84 y 112 kg N/ha. El uso de niveles de fertilización mayores resultará en un menor beneficio económico y mayor potencial de contaminación ambiental.

Palabras clave: Líneas puras de maíz, coberturas, fertilizante-N, respuesta a la fertilización

INTRODUCTION

Maize (*Zea mays* L.) is an important commodity crop that provides protein, oil, and starch for food, animal feed, ethanol, and other bio-based products. The global area of maize production was estimated at 159.5 × 10^6 ha and 32.2 × 10^6 ha in the United States in 2009 (FAOSTAT, 2011). An annual grain yield increase of 125 kg/ha/yr in developed countries is attributed to the use of advanced agronomic management including fertilizers and improved genetics. Maize in Puerto Rico has traditionally been used for animal feed and to a lesser extent for fresh-market consumption, with local production accounting for only a small portion in terms of volume and economic value. The genetic materials that have been traditionally grown in Puerto Rico are open pollinated cultivars such as 'Diente de Caballo', and 'Mayorbela' (USDA-ARS, 1990), 'Mayorbela 05' (Beaver et al., 2006), 'Chulo' (USDA-ARS, 1996), sweet corn 'Suresweet' (USDA-ARS, undated), and commercial hybrids. Research has shown that with technological advances and adequate pest control, excellent commercial yields can be achieved for maize production in Puerto Rico (Sotomayor-Ríos, 1980; Sotomayor-Ríos et al., 1984; Vicente-Chandler, 1993).

During the past three decades, commercial winter nurseries and agro-biotechnology firms have been established in Puerto Rico, primarily on the southern semi-arid coast, in fields that were previously under sugarcane (*Saccharum officinarum* L.) production. Maize is grown for seed production, seed propagation, and hybrid selection in a production area estimated at 2,300 ha, distributed among seven major companies. Maize is grown primarily from November to March, in monoculture. When not under cultivation, the soils are kept weed-free by disking and other tillage operations. There is concern that current cropping practices may have a negative impact on soil quality and on agro-ecosystem sustainability. The use of cover crops has multiple benefits including reduced fertilizer inputs, less need for herbicides, improved seed yields as a result of enhancing soil health, less soil erosion, conserved soil moisture and improved water quality (Delgado et al., 2007; SAN, 2007). An evaluation of the agronomic performance of cover crops in this area has been reported elsewhere (Sotomayor-Ramírez et al., 2009), yet the effects of cover crops on maize production have not been reported.

Fertilizer-nitrogen (N) continues to be the most frequent limiting factor in maize cropping systems, and considerable uncertainty exists regarding the optimum fertilizer-N application because of variations in soils, climatic factors, expected seed yields of genetic materials, and fertilizer recommendation philosophies (Vanotti and Bundy, 1994; Wilhelm et al., 1995; Fixen, 2006). At the local level, thirteen publications and various Master of Science theses have been published dealing with fertility/nutrient management aspects of maize. None of these publications have addressed fertilizer-N response or requirements of maize inbreds.

Inbred parental seed is the basic building block for the production of hybrids. Inbred lines typically are shorter, have less vigor, thinner stalks, smaller tassels, smaller ears, and lower seed yields compared to open-pollinated maize varieties or hybrids. A possible decreased rooting capacity makes inbreds more vulnerable to nutrient imbalances and deficiencies, drought stress, diseases and insects. In an effort to obtain higher seed yields, fields of inbred maize are frequently overfertilized. Beck (2002) suggested that best seed yields can be achieved with lower N rates (55 to 110 kg/ha) than that recommended by many companies and seed producers. Wilhelm et al. (1995) reported average optimum fertilizer-N rates of 86 kg N/ha for various inbred maize lines.

In general, macronutrient levels (P, K, Ca, Mg) in soils of the southern semiarid coast of Puerto Rico are above soil test critical levels (Sotomayor-Ramírez and Martínez, 2006), which suggests that only maintenance application of most nutrients is warranted. However, site-specific information has not been gathered to prove that plant response to specific nutrients does not occur. Plant response to N fertilization is expected to occur because little N accumulates in the soil profile in inorganic form. The maize inbreds are planted after the rainy season, when most residual profile N leaching is expected to have occurred, and low soil organic matter levels impede accumulation of large N reserves in organic form. The overall objective of this paper is to provide information to improve fertilizer-N management in inbred-maize production systems in Puerto Rico. Specifically we wanted to: (i) determine maize seed yield response to fertilizer-N addition and cover crop management; (ii) evaluate some agronomic predictors of optimum nutrient status; (iii) evaluate potential N losses in the inbred maize production systems.

MATERIALS AND METHODS

The study site was the Mycogen Seeds Corp.[7] research farm in Santa Isabel, Puerto Rico. The predominant soil series within the farm are Jacaguas (Loamy-skeletal, mixed, superactive, isohyperthermic Fluventic Haplustolls) and Fraternidad (Fine smectitic, isohyperthermic Typic Haplusterts) (USDA-SCS, 1979). Two field trials were performed. Trial 1 evaluated the effects of two cover crops and five fertilizer-N levels on maize seed yield response, and trial 2 evaluated the effect of five fertilizer-N levels on maize seed yield.

In trial 1, velvet bean (*Mucuna prurience)* and cowpea (*Vigna unguiculata*) ‘Iron clay’, were grown as cover crops during the summer of 2008. A fallow area adjacent to the cover crops was also included in the trial and was maintained by the application of glyphosate and shallow disking. At the end of the growing period, both cover crops were sprayed with glyphosate and incorporated via disking into the soil in late September 2008. Soil preparation prior to corn planting (during October and November 2008) included chisel plowing and disking.

Corn (inbred maize line A[8]) was sown 10 December 2008. Within each cover crop-fallow treatment, corn response to fertilizer-N application was evaluated, with four replications. The experimental design was a split-plot arrangement of a RCB with previous cover crop as the main plot and N level as the sub-plot. The sub-plots had an area of 42 m^2 (4.6 × 9.1 m) with six rows (spaced 0.76 cm apart) per sub-plot.

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[7] Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

[8] Inbred identification Lines have been omitted because of Dow AgroSciences policy.

Final crop density for all treatments was on average 52,090 plants/ha. The fertilizer-N levels consisted of 0, 112, 150, 186, and 228 kg N/ha. All of the fertilizer-N treatments, except the control (N1) received 50 kg N/ha via sub-surface band at planting. A fertilizer-N surface band side-dressing of ammonium sulfate was applied 30 days after planting (DAP) (just prior to the six-leaf stage) to achieve final rates of 112, 150, 186, 228 kg N/ha. At planting, all subplots received 67, 112, and 28 kg/ha of P_2O_5, K_2O and ME (minor elements), respectively. The sources of P, K, and ME were a fertilizer mix of triple superphosphate, muriate of potash, and Five-Star-Mix®, respectively, which was broadcast prior to planting. Irrigation was provided by drip irrigation following farm practices.

Agronomic data was gathered at 40, 54, and 70 (silking) DAP, and included plant height, leaf area index (LAI), and SPAD color index. SPAD-502 chlorophyll meter (Minolta Corp.) readings were taken from the youngest leaf with fully expanded collar at 40 and 54 DAP, and from the leaf opposite the top ear near silking. This leaf was also sampled for N sufficiency status. Plant greenness was assessed with an IRRI Leaf Color Chart (scale of 1 to 4) at 54 DAP. The LAI was taken with a LICOR-2000 (Li-Cor, Lincoln NE) (Li-Cor, 1992) from dawn until 8:00 AM (maximum) as specified by Malone et al. (2002). Plant nutrient (N, P, K, Ca, Mg, S) concentrations were determined from indicator leaves at 70 DAP. At maturity (90 DAP), plant biomass (leaves, stems and husks) and nutrient content, and corn grain yield were determined.

In field trial 2, the field was under continuous corn cultivation (winter-early spring) followed by a fallow period (from late spring to fall) every year. The experimental design was a RCBD with five fertilizer N levels (0, 84, 125, 168, 211 kg N/ha) and four replications. Each main plot was 19.8 m long and consisted of six rows with a 0.76 cm inter-row spacing. A five-foot border separated the two main-plots, and two unfertilized maize rows separated the sub-plots. Corn (inbred maize line B) was sown 26 February 2010. Fertilizer-N was applied at 7 DAP at a rate of 28 kg N/ha with half of the N applied as $(NH_4)_2SO_4$-N and the other half as KNO_3-N. One-third of the remaining amount of N for each treatment was applied at 28 DAP (V4) in proportion 1:1 of $(NH_4)_2SO_4$-N and KNO_3-N. Two-thirds of the remaining amount of N was applied at 49 DAP (VT) in proportions 2:1 of $(NH_4)_2SO_4$-N and KNO_3-N. All fertilizer-N was applied by fertigation. All plots received 67, 112, and 28 kg/ha of P_2O_5, K_2O and ME, respectively, in the same forms as in Trial 1.

Irrigation was provided by drip irrigation following farm practices. Agronomic data gathered included SPAD readings, plant height and leaf color as described in Trial 1. In Trial 2, SPAD-502 chlorophyll meter (Minolta Corp.) readings were taken at 33 DAP (V5) and 54 DAP (R1, near silking). Leaf plant nutrient sufficiency status was taken at 56 DAP (R1). Plant greenness was assessed as described previously at 33 DAP (V5, 31 March 2010) and 54 DAP (R1). Plant biomass (leaves, stems and husks) and nutrient content were determined at 77 DAP, and corn seed yields were determined at 91 DAP. For both trials, seed yields were expressed at 15.5% moisture, and nutrient efficiency indicators were quantified as described below.

Nutrient use efficiency was measured for a given genotype as the ability to produce a unit of seed yield per unit of nutrient applied (Baligar et al., 1990). There are two main ways to calculate nutrient use efficiency. One is based on the difference method, and the other on the balance method (Syers et al., 2008). Three indicators were calculated by using the difference method. The Agronomic Efficiency (AE_Y-DM) is defined as the increase in seed yield obtained per unit of nutrient applied. It can be calculated as:

$$AE_Y\text{-DM} = (YG_f - YG_u)/N_a \qquad [1]$$

where, YG_f, YG_u, and N_a are seed yield in fertilized plants, seed yield in unfertilized plants, and nutrient applied, respectively.

The same index can be calculated by using the biological yield (BY) (seed + straw), and is defined as the increase in whole plant biomass per unit of nutrient applied (AE_{BY}-DM):

$$AE_{BY}\text{-DM} = (BY_f - BY_u)/N_a \qquad [2]$$

The Apparent Recovery Efficiency (ARE-DM) is defined as the increase in whole plant nutrient uptake per unit of nutrient applied. It is also known as the apparent nutrient recovery (ANR) (Baligar et al., 1990) and is defined as:

$$\text{ARE-DM} = (NBY_f - NBY_u)/N_a \qquad [3]$$

where NBY and NBY_u are the whole plant biomass N in fertilized and unfertilized plants, respectively.

Three indicators were calculated by using the balance methods. The partial factor productivity (PFP-BM) (Syers et al., 2008) is defined as the amount of grain harvested per unit of N applied:

$$\text{PFP-BM} = YG_f/N_a \qquad [4]$$

At the optimum value, it can be used for making fertilizer recommendations based on yield goal.

The partial balance productivity (PBP-BM) (Syers et al., 2008) is defined as the amount of nutrient removed in grain (NG_f) per unit of N applied. It can serve as an indicator of the proportion of N applied that is removed in the grain as:

$$\text{PBP-BM} = NG_f / N_a \qquad [5]$$

It can be used for making fertilizer recommendations based on N removal.

The agronomic efficiency (AE-BM) based on the balance method is defined as the amount of nutrient removed in the whole crop per unit of N applied.

$$AE = NBY_f / N_a \qquad [6]$$

It can serve as an indicator of excess or deficit N in the system.

All leaf and vegetative biomass material was washed with tap water, dried and sent to a commercial laboratory (MDS Harris Laboratories®) for quantification of total N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and B. Soils were sampled for soil fertility parameters at 0- to 15-cm depth prior to corn planting. Results were similar for both trials; thus only the results for trial 1 are included (Table 1).

Statistical analyses were performed separately for the two trials. All data were subjected to analysis of variance by using Proc Mixed of SAS (SAS Institute, Cary, NC). The reported treatment means are the LSMeans output of the model when the main effects or interaction were significant. Contrasts were used to make comparisons between the control (no fertilizer-N applied) and the other fertilizer N levels. When appropriate, multiple comparisons among treatments were made with Fisher's Least Significant Difference test. When no significant difference among means for a particular variable was observed, the mean and standard error were presented. Correlation analysis was done by using the CORR procedure in SAS.

RESULTS

Trial 1. Fertilizer-N was the most important main effect influencing ($P \leq 0.05$) maize agronomic traits, with cover crop affecting only four of these (Table 2). The fertilizer-N x cover crop interaction was significant only for leaf color index (54 DAP) and LAI (40 DAP), but these were not considered important due to the high fertilizer-N main effect significance and the trends observed for other parameters. Fertilizer N did not significantly affect the number of ears or grain N concentration with mean values of 67,099 ears/ha and 1.72% N, respectively.

TABLE 1.—*General soil fertility parameters*[1] *at the study site in trial 1 (2008-2009). Samples were taken after cover crop planting and before maize cropping.*

	pH	OM	NO_3-N	Extractable P	Ca	Mg	K	CIC	Fe	Mn	Zn	Cu
Cover crop		%	mg/kg		cmolc/kg				mg/kg			
Velvet bean	7.6	2.51	18.4	21.8	24.5	6.2	0.60	31.6	6.4	1.5	2.1	6.6
Cowpea	7.6	2.45	17.2	23.9	24.8	6.3	0.62	32.0	5.9	1.2	1.6	5.6
Fallow	7.8	2.52	13.8	22.4	26.7	6.3	0.57	33.9	5.0	1.1	1.2	5.8
Mean	7.7	2.49	16.5	22.7	25.3	6.3	0.60	32.5	5.8	1.3	1.6	6.0
Soil fertility level[2]	H	M	M	H	H	H	H	H	H	H	H	H

[1] pH was measured in 1:2 soil water ratio; OM is soil organic matter measured by weight loss on ignition; NO_3-N is 1M KCl extractable; extractable P is that using the Olsen-P method; Ca, Mg, K were extracted by ammonium acetate and quantified by ICP; CIC is the cation exchange capacity; Fe, Mn, Zn and Cu were extracted with DTPA-TEA.

[2] H is high; M is medium; L is low fertility classification as described in Sotomayor-Ramírez and Martínez (2006).

TABLE 2.—*Summary of ANOVA to examine the effect of cover crop and fertilizer N levels, and contrasts between the control and fertilized plots, for various agronomic parameters of inbred maize during 2008-2009 (Trial 1).*

					Fertilizer N		Unfertilized (control)	
Effect	CC[1]	N Level[1]	CC* N Level	Contrast between control and others (significance level)	Mean	Standard error	Mean	Standard error
Seed yield (kg/ha	**[2]	**	ns	***	2,726	87.3	1,788	177.5
Stover wt (kg/ha)	ns	***	ns	***	6,620	107.4	5,690	178.6
Ears/ha	ns	ns	ns	ns				
Harvest index	**	**	*	***	0.26	0.01	0.21	0.01
Plant biomass (grain + stover) dry wt (kg/ha)	ns	***	ns	***	9,346	151.2	7,474	294.1
Stover N (kg/ha)	ns	***	ns	***	107.9	1.9	83.0	3.8
Grain N concentration (%)	ns	ns	ns	ns				
Grain N uptake (kg/ha)	*	**	ns	***	39.7	1.5	25.7	3.0
Crop (stover + grain) N uptake (kg/ha)	ns	***	ns	***	149.3	2.8	110.4	5.8
SPAD chlorophyll reading (40 DAP)	ns	***	ns	***	50.6	0.4	43.1	0.7
SPAD chlorophyll reading (54 DAP)	ns	***	ns	***	53.4	0.4	50.7	0.5
SPAD chlorophyll reading (70 DAP)	**	**	ns	***	54.3	0.2	52.6	0.5
Leaf color index (54 DAP)	ns	***	**	***	4.2	0.1	3.3	0.1
LAI (40 DAP)	ns	***	***	***	1.05	0.02	0.82	0.04
LAI (54 DAP)	ns	**	ns	***	1.71	0.03	1.47	0.05
LAI (70 DAP)	ns	**	ns	***	1.64	0.03	1.40	0.06
Plant height (cm), (40 DAP)	***	***	ns	***	33.8	0.4	26.4	0.7
Plant height (cm), (54 DAP)	ns	***	ns	***	59.5	0.8	48.0	1.1
Plant height (cm), (70 DAP)	ns	**	ns	***	64.2	1.1	59.9	1.3

[1] CC is cover crop; N level is fertilizer N treatment.

[2] *, **, ***; indicates significance at $P \le 0.1$, 0.05, and 0.001, respectively; ns denotes that no significant $P>0.1$ main effect or interaction effects were detected.

Maize seed yield was significantly affected by cover crop and fertilizer-N (Table 2). Maize yield (kg/ha) was highest following velvet bean (2,903) and cowpea (2,522) and the latter was similar to that after fallow (2,189). Harvest index followed the same trend as observed for grain yield, with highest values for maize grown after velvet bean (0.27) and cowpea (0.25) and the latter was similar to that after fallow (0.22). Maximum seed yield was obtained with 112 kg N/ha, with no significant difference among the higher fertilizer-N levels. Seed yield was 2,726 kg/ha (mean of four fertilizer-N levels) with fertilizer N, and 1,788 kg/ha without fertilizer N.

Fertilizer-N rates of 112 to 224 kg/ha did not result in significant differences in stover weight, harvest index, plant biomass, stover N, grain N uptake, or crop N uptake, but these were higher than those of the control (Table 2). Fertilizer-N rates of 112 to 224 kg/ha did not result in significant differences in indicator-leaf SPAD chlorophyll index at all stages of crop development, but these were higher than in the control (Figure 1). Indicator-leaf SPAD chlorophyll index increased

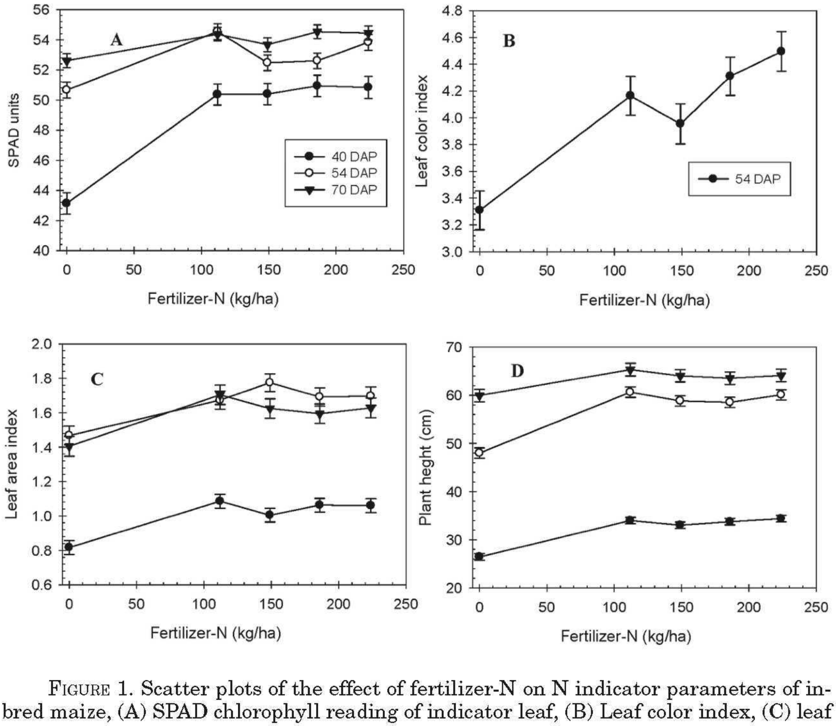

FIGURE 1. Scatter plots of the effect of fertilizer-N on N indicator parameters of inbred maize, (A) SPAD chlorophyll reading of indicator leaf, (B) Leaf color index, (C) leaf area index, (D) plant height, during 2008-2009 (Trial 1).

with crop development stages from 40, 54 and 70 DAP with mean values in fertilized plants of 50.6, 53.4, and 54.3, and mean values in unfertilized plants of 43.1, 50.7, and 52.6, respectively. Indicator-leaf color at 54 DAP was significantly higher in plants receiving fertilizer N than in the control. Plant height at all stages of crop development was significantly higher in treatments receiving fertilizer N than in the control. Plant height increased with crop development stages of 40, 54 and 70 DAP with mean values (cm) in fertilized plants of 33.8, 59.5, and 64.2, respectively, and mean values in unfertilized plants of 26.4, 48.0, 59.9, respectively.

Cover cropping improved indicator maize leaf nutrient concentration of N (3.32% with cover crops, versus 3.17% without cover crops) and S (0.43% for cowpea and fallow versus 0.38 for velvet bean) (Table 3). The effect of fertilizer N was significant only for Mg (0.19 in unfertilized maize and 0.17% in fertilized maize) and Mn concentrations. The interaction fertilizer x cover crop affected leaf Fe concentration. Contrary to what was expected, fertilizer-N treatments did not influence indicator leaf N concentration, with a mean value of 3.27%.

Trial 2. Maize seed yield was significantly higher ($P \leq 0.05$) in fertilized treatments (1,447 kg/ha) than in the control (1,181 kg/ha) (Tables 4 and 5). No significant treatment effects were observed for stover dry-matter biomass, number of ears, harvest index, crop (grain + stover) biomass, stover N uptake, stover P uptake, stover K uptake, grain N concentration, crop (grain + stover) N uptake, crop (grain + stover) P uptake, crop (grain + stover) K uptake. Grain nutrient uptake was significantly higher ($P \leq 0.05$) in fertilized treatments as compared to the control, with mean values for the fertilized treatments for N, P, and K

TABLE 3.—*Nutrient concentrations of indicator leaf of inbred maize during 2008-2009 (Trial 1) and 2009-2010 (Trial 2).*

	2008-2009 (Trial 1)		2009-2010 (Trial 2)	
Variable	Mean	Standard deviation	Mean	Standard deviation
N (%)	3.27	0.15	—[1]	
P (%)	0.36	0.02	0.40	0.00
K (%)	2.04	0.09	2.41	0.05
Ca (%)	0.37	0.03	0.60	0.01
Mg (%)[2]	0.17	0.03	0.24	0.02
S (%)	0.42	0.04	0.27	0.00
Fe (mg/kg)[3]	142.7	34.1	62.3	2.2
Mn (mg/kg)[2]	103.7	29.2	72.0	2.0
Zn (mg/kg)	18.3	2.1	16.4	0.6
Cu (mg/kg)	14.2	1.7	—[1]	
B (mg/kg)	10.1	4.7	29.4	1.4
Mo (mg/kg)	1.9	1.0		

[1] N and Cu concentrations for Trial 2 are discussed in the text.
[2] Mean of fertilized treatments only for 2009.
[3] Fe concentrations were affected by fertilizer x cover crop interaction for 2009.

TABLE 4.—*Summary of ANOVA to examine the effect of fertilizer N levels and of contrasts between the control and fertilized plots, for various agronomic parameters of inbred maize during 2009-2010.*

Effect	Pr > F	Contrast between control and others (significance levels)	Mean[2]	Standard error
Seed yield (kg/ha	ns[1]	**	n/a	
Stover wt (kg/ha)	ns	ns	4,567	216.4
Ears/ha	ns	ns	74,599	1,115.9
Harvest index	ns	ns	0.269	0.017
Plant biomass (grain + stover) dry wt (kg/ha)	ns	ns	5,958	213.2
Stover N (kg/ha)	ns	ns	51.3	3.4
Stover P (kg/ha)	ns	ns	13.3	0.8
Stover K (kg/ha)	ns	ns	99.0	5.4
Grain N concentration (%)	ns	ns	1.46	0.03
Grain N uptake (kg/ha)	ns	*	n/a	
Grain P uptake (kg/ha)	ns	**	n/a	
Grain K uptake (kg/ha)	*	**	n/a	
Crop (stover + grain) N uptake (kg/ha)	ns	ns	68.3	3.4
Crop (stover + grain) P uptake (kg/ha)	ns	ns	17.8	0.8
Crop (stover + grain) K uptake (kg/ha)	ns	ns	103.8	5.4
SPAD chlorophyll reading (V5)	ns	ns	44.6	0.9
SPAD chlorophyll reading (R1)	*	***	n/a	
Leaf color index (V5)	**	**	n/a	
Leaf color index (R1)	*	*	n/a	
Plant height (cm), (V5)	ns	ns	13	0.4
Plant height (cm), (R1)	ns	ns	39	0.4

[1] *, **, ***; indicates significance at $P \leq 0.1$, 0.05, and 0.001, respectively; ns denotes that no significant ($P > 0.1$) main effect or interaction effects were detected.

[2] The mean value represents that of all treatments when contrast effects were non-significant ($P > 0.1$).

TABLE 5.—*Treatment means of selected agronomic parameters of inbred maize during 2009-2010. Only parameters in which there was a significant fertilizer-N level effect are included.*

N level	Seed yield	Seed N uptake	Seed P uptake	Seed K uptake
	- - - - - - - - - - - - - -	kg/ha	- - - - - - - - - - - - - -	
0	1,181 b[1]	14.7 b	3.8 b	3.9 b
84	1,449 ab	17.0 ab	4.9 a	5.2 a
125	1,308 ab	16.1 ab	4.5 ab	4.7 ab
168	1,522 a	18.3 ab	5.1 a	5.3 a
211	1,495 a	19.3 a	4.5 ab	5.3 a
	- - - - - - - - - - - - - -	Mean of fertilized treatments	- - - - - - - - - - - - - -	
	1,446.8	17.6	4.7	5.1

[1] Treatment means with different letters are significantly different as determined with Fisher's Least Significance Difference test (P ≤ 0.05).

of 17.6, 4.7, and 5.1 kg/ha, respectively, and for unfertilized treatments for N, P, and K of 14.7, 3.8, and 3.9 kg/ha, respectively.

Although the SPAD chlorophyll reading taken from the indicator leaf at V5 growth-stage did not identify treatment differences, the same variable at R1 growth-stage adequately separated out the control (mean of 49.0) from the fertilized plots (mean of 51.2) (Table 6). The leaf color index taken at both V5 and R1 growth stages significantly identified treatment differences between the control and the fertilized plots. The mean value in the control was 3.05 and 3.34 at V5 and R1 in the control plots, and 3.41 and 3.56 in the fertilized plots, respectively.

TABLE 6.—*Treatment means of selected N indicator parameters of inbred maize during 2009-2010. Only parameters in which there was a significant fertilizer-N level effect are included.*

N level	SPAD (R1)	Leaf color index (V5)	Leaf color index (R1)
kg/ha			
0	49.00 b[1]	3.05 c	3.34 b
84	51.05 a	3.16 bc	3.41 b
125	50.78 ab	3.22 bc	3.54 ab
168	51.43 a	3.54 ab	3.72 a
211	51.33 a	3.70 a	3.58 ab
	- - - - - - - - - - - - - -	Mean of fertilized treatments	- - - - - - - - - - - - - -
	51.14	3.41	3.56

[1] Treatment means with different letters are significantly different (P ≤ 0.05) as determined with Fisher's Least Significance Difference test.

Leaf SPAD readings and Leaf Color Index values were slightly lower than those reported for Line A during 2009 at 54 DAP.

Leaf nutrient sufficiency levels were not affected by the treatments, except for N and Cu, in which case the contrast between the control and the fertilized plots was possibly significant ($P < 0.1$) for N, and significantly different ($P < 0.05$) for Cu (Table 3). Mean N concentrations in the control and fertilized plots were 2.14 and 2.32%, respectively. Mean Cu concentrations in the control and fertilized plots were 17.8 and 16.6 mg/kg, respectively.

Nitrogen use efficiency. In 2009, the effect of fertilizer N levels was observed for all nutrient use efficiency indicators. In general, N use efficiency decreased with increasing fertilizer-N applied. In 2010, fertilizer-N application did not have a significant effect on AE_{BY}-DM or ARE-DM, where mean values of 1.23 and 0.045 were obtained, respectively (Table 7). In 2010, the effect of fertilizer N application was observed for AE_Y-DM, PFP-BM, PBP-BM, and AE-BM ($P < 0.05$). The highest N use efficiency was observed for the 84 kg N/ha fertilizer level for all of the indicators. Nitrogen use efficiency decreased with increasing fertilizer-N applied.

TABLE 7.—*Treatment means of selected N use efficiency indicator parameters*[1] *of inbred maize during 2008-2009 and 2009-2010. Only parameters in which there was a significant fertilizer-N level effect are included.*

N level	AE_Y-DM	AE_{BY}-DM	ARE-DM	PFP-BM	PBP-BM	AE-BM
2008-2009						
112	9.1 a[2]	16.9 a	0.35 a	24.4 a	0.35 a	1.32 a
149	6.2 ab	10.5 ab	0.21 b	17.6 b	0.26 b	0.93 b
186	5.7 ab	9.3 b	0.22 ab	14.9 bc	0.22 bc	0.81 c
224	4.9 b	8.4 b	0.20 b	12.6 c	0.18 c	0.69 c
2009-2010						
84	3.4 a	ns[3]	ns[3]	17.5 a	0.21 a	0.81 a
125	0.7 b			10.2 b	0.13 b	0.53 b
168	1.9 ab			9.0 bc	0.11 bc	0.44 b
211	1.5 ab			7.1 c	0.09 c	0.36 b

[1] AE_Y-DM is the agronomic efficiency in yield by the difference method; AE_{BY}-DM is the agronomic efficiency in seed and stover by the difference method; ARE-DM is the apparent recovery efficiency by the difference method; PFP-BM is the partial factor productivity by the balance method, PBP-BM is the partial balance productivity by the balance method, and AE-BM is the agronomic efficiency by the balance method.

[2] Treatment means with different letters are significantly different ($P \leq 0.05$) as determined with Fisher's Least Significance Difference test.

[3] ns, treatment effects were not significant ($P > 0.05$).

DISCUSSION

During 2008-2009 seed yield for Line A was double and for Line B about 26% lower than reported acceptable yields for the same materials in the area. We estimate that the yield loss reduction in 2010 was due to the incidence of corn ear-worm (*Helicoverpa zea*) and, to a lesser extent to fall army-worm (*Spodoptera frugiperda*) damage. Therefore, 20 to 30% higher grain yields could potentially be obtained than those reported here under similar soils and climatic conditions with improved pest control.

For 2009-2010, nutrient uptake values indicate that relatively low amounts of P and K were removed from the field during harvest. The crop (stover + grain) removed 18 and 104 kg/ha, of P and K, respectively, of which about 26% of P and 5% of K is removed in the grain. Crop removal uptake data for P and K in 2008-2009 was unavailable. When no crop response to fertilizer nutrient application is expected, application rates should be in close balance with crop removal to ensure maximum efficiency and minimum environmental impact due to gaseous losses, leaching and runoff. The values determined in this experiment can serve as a general guideline for future P and K management. However, further work should examine crop response to fertilizer P and K.

Seed yields in 2008-2009 were significantly correlated ($P < 0.05$) with certain yield components (stover biomass, harvest index, plant biomass, stover-N, grain-N uptake, and crop N uptake), and with N use efficiency indicators in 2009 (Data not shown). Most of these are post-harvest and destructive. Therefore, the value of using them as diagnostic tools for N sufficiency may be in identifying these traits and selecting for those that are desirable in corn inbreds. Grain yields were significantly correlated ($P < 0.05$) with crop agronomic indicators: SPAD chlorophyll meter, LAI, plant height, and leaf color. Since the SPAD chlorophyll meter readings and plant color are probably the easiest variables to measure, for Line A, leaf color index of 4.2 at 54 DAP and SPAD chlorophyll meter readings of 50.6, 53.4, and 54.3 can be used as N sufficiency indicators at 40, 54 and 70 DAP. Similarly for Line B, leaf color index of 3.41 and 3.56 at V5 and R1, respectively, and SPAD chlorophyll meter readings of 44.6 and 51.2 at V5 and R1, respectively, can be used. Indicator leaf N concentration of 3.31% for Line A and of 2.14% for Line B could be used as sufficiency values. The applications of fertilizer N beyond the optimum response levels at the specified growth stage will not result in improved crop yields.

Fertilizer N improved the harvest index (partitioning between seed and total plant biomass) in 2009, but not in 2010. The harvest index in fertilized maize in 2009 and in 2010 was similar. An important contributor to the lower seed yields of inbreds may be the low harvest index characteristics.

Typical fertilizer-N recommendations for hybrid maize based on expected yield is 20 kg N/1,000 kg grain, yet this approach does not account for soil N availability, N supply during the growing season, or other internal physiological factors influencing N uptake. On the basis of this rationale, with seed yields obtained in 2009 and 2010, fertilizer N recommendation would be 56 and 29 kg N/ha, respectively. Said fertilizer N levels would have been sufficient (i.e., between 41 and 65% in excess of that removed) to account for N export in grain with optimum yields (estimated at 39.7 kg/ha in 2009 and 17.6 kg/ha in 2010) and to maintain an adequate N balance in soil. With fertilizer-N recommendations of 56 and 29 kg N/ha, a substantial amount of the total N used by the crop (estimated at 149 kg N/ha in 2009 and 68 kg N/ha for optimum yields) must come from the soil residual NO_3-N, and from soil N mineralization. Our empirical estimate of soil residual NO_3-N and soil N mineralization (based on crop N extraction from unfertilized maize) was 110 kg N/ha in 2009 and 64 kg N/ha in 2010. These values are an approximation as crop N uptake is influenced by climatic factors affecting the soil N mineralization process (Rice and Havlin, 1994) and crop genetic factors. Clearly, the optimum fertilizer-N levels of 112 and 84 kg N/ha found in these experiments were sufficient to account for the N removed in grain and to optimize yields, but it is not known if lower fertilizer-N levels may provide the same benefit. Under similar agronomic and climatic conditions and maize inbreds, it may be possible that the lowest fertilizer-N levels tested in these experiments could be sufficient to maintain yields and cropping system sustainability.

Potentially available N from previous velvet bean and cowpea cover crops was estimated at 101 and 144 kg N/ha, respectively (Sotomayor et al., 2009). However, crop N uptake was not influenced by cover crop or cover crop x fertilizer-N interaction. We presume that residual soil N from previous velvet bean or from cowpea was either inmobilized as organic matter in labile form or was lost from the system, and was therefore not directly and immediately available to the subsequent maize crop. Improved benefit of N availability from the cover crop will occur with the appropriate timing of the cover crop vegetative mineralization process with subsequent maize cropping.

Consideration of the fertilizer-N levels tested and the crop and grain removal values determined, residual soil N after maize cropping increased with fertilizer-N level applied, and was estimated at between -37 and 68 kg N/ha in 2009, and between 14 and 136 kg N/ha in 2010. Stover N decomposition could add 108 and 52 kg N/ha for 2009 and 2010, respectively. Thus the combination of residual soil N and stover N decomposition would add between 67 and 192 kg N/ha (increased with fertilization level) to the soil and subsequent cropping. These values could be considered as those which could potentially be lost from the agroecosystem between cropping if soils are left fallow.

The highest nutrient efficiency indicators were obtained with 84 and 112 kg N/ha, for 2010 and 2009, respectively; these were not affected by cover crop or fertilizer-N x cover crop interaction. The indicators AE_{BY}-DM and ARE-DM were not affected by fertilizer-N in 2010. On average, 9.1 and 3.4 kg additional grain (AE_{BY}-DM) was produced per kilogram of fertilizer-N applied up to 112 and 84 kg N/ha in 2009 and 2010, respectively. Under optimum conditions agronomic efficiencies of hybrid corn, as measured by AE_{BY}-DM, are 30 kg grain/kg N applied. In this study, an optimum PFP-BM value of 24.4 in 2009 and 17.5 in 2010 was obtained. Recommended PFP values for hybrid corn are between 40 and 80 with a mean value of 50 in Nebraska (Doberman, 2008 as cited by Fixen, 2009). Nitrogen agronomic efficiency as expressed in terms of PFP values has been consistently improving in the United States with values of between 60 and 70 from 2000 to 2010, so that higher yields can be achieved per unit of N applied. This progress is a result of improvements in fertilizer management, the use of diagnostic nutrient sufficiency indices, crop management practices and crop genetic improvement (Snyder, 2009). The inverse of our reported PFP values indicates that between 41 and 57 kg N/ha are needed to produce 1,000 kg grain/ha. In inbred lines with characteristics similar to the ones evaluated, the PFP value can be used to guide fertilizer-N management, and is substantially different than for hybrid corn.

Maximum PBP-BM values of 0.35 and 0.21 indicate that between 29 and 48 kg fertilizer-N are needed for every 10 kg N removed in the grain in order to maintain an adequate N balance because of grain N removal from the field. Values < 1 are indicative of excess N, and optimum values should approach 1. Crop managers can use the information to improve their field-by-field fertilizer N budgeting.

CONCLUSIONS

This paper reports on seed yield response of inbred maize to fertilizer N and cover crops, under specific crop management practices and specific fertilizer N sources, location, and timing. The results obtained may change because of variations in inbred lines used, and fertilizer and crop management patterns. The information can be used to quantitatively guide fertilizer-N management on the basis of each manager's philosophical approach. The specific options for making specific fertilizer N management decisions are beyond the scope of this work and will be addressed elsewhere. For example, a producer may decide to manage fertilizer N based on crop yield response only, or in combination with other aspects such as grain/fertilizer price ratios, pre-plant soil NO_3 content, expected yields and efficiency estimates. The results of this study demonstrate that fertilizer N management of inbreds should be different from that for hybrid corn. Application of fertilizer-N beyond crop response will not result in agronomic benefit.

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