Nitrogen fertilizer rate for improving inbred maize (*Zea mays* L.) yield on the semi-arid southern coast of Puerto Rico[1,2]

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ABSTRACT

Nitrogen (N) is possibly the most limiting nutrient for crop production on the southern semi-arid coast of Puerto Rico. In efforts to improve inbred maize (*Zea mays* L.) grain yield, fertilizer N is sometimes aggressively managed. In this paper, we report on the results of a field experiment that evaluated the effect of six rates of fertilizer N (0, 34, 68, 102, 135 and 203 kg N/ha) and of cowpea (*Vigna unguiculata* cv. Iron-clay), planted as a cover crop during the offseason, on inbred maize grain yield. The soil was Jacaguas series (Loamy-skeletal, mixed, superactive, isohyperthermic Fluventic Haplustolls) on the Dow Agrosciences experimental farm in Santa Isabel, Puerto Rico. Cowpea was planted on 13 July 2013 and incorporated into the soil on 20 September 2013. An inbred maize line was planted on 19 December 2013 and harvested on 19 March 2014 at a plant density of 51,645 plants per hectare. Irrigation was provided via drip system, and fertilizer N was applied at three different stages during the growing season: at emergence, 21 and 37 days after planting. Measurements of plant height, chlorophyll readings using SPAD-502® and GreenSeeker®, and leaf N concentration were used as indicators of treatment response and N sufficiency. The maximum grain yield of 2,918 kg/ha was attained with the fertilizer N rate of 68 kg N/ha. The cowpea cover crop rotation did not affect grain yield ($P>0.05$). Plant height, and measurements by SPAD-502® and GreenSeeker® provided adequate indicators of crop N sufficiency during the vegetative stages V6 to V12, with optimum values of 149 cm, 46, and 0.67 NDVI, respectively, 52 days after planting with an application of 68 kg N/ ha. Crop response to fertilizer N occurred at a lower rate than in previous studies and those occurring under conventional commercial conditions. Other factors related to fertilizer N management, such as sources, placement and timing of application might be as important for grain yield improvement of inbred maize.

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

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RESUMEN

Nivel de fertilizante nitrogenado para mejoramiento del rendimiento de una línea pura de maíz en la zona semiárida de la costa sur de Puerto Rico

El nitrógeno (N) es posiblemente el nutriente más limitante para la producción de cultivos en la costa semiárida sur de Puerto Rico. Usualmente el fertilizante-N es manejado agresivamente en esfuerzos para mejorar la producción de semillas endogámicas de maíz (*Zea mays* L.). En este artículo presentamos los resultados de un experimento de campo que evaluó el efecto de seis niveles de fertilizante N (0, 34, 68, 102, 135, 203 kg/ha) y caupí (*Vigna unguiculata* cv. Iron-clay) como cobertura en rotación, en el rendimiento de semilla de maíz endogámico. El experimento se realizó en un suelo de la serie Jacaguas (*Loamy-skeletal, mixed, superactive, isohyperthermic Fluventic Haplustolls*) en la finca de DowAgrosciences® en el municipio de Santa Isabel, Puerto Rico. La siembra de la cobertura fue el 13 de julio de 2013, y se incorporó al suelo el 20 de septiembre de 2013. El maíz se sembró el 19 de diciembre de 2013 a una densidad de 51,645 plantas por hectárea, y se cosechó el 19 de marzo de 2014. El riego se realizó mediante un sistema de goteo y el fertilizante N se aplicó durante la emergencia, 21 y 37 días después de la siembra. El desarrollo del cultivo se evaluó midiendo la altura de la planta y el área foliar de una hoja indicadora. Los valores provistos por los instrumentos GreenSeeker®, SPAD-502® y la concentración de N en la hoja índice se evaluaron como indicadores de suficiencia de N en el cultivo. Se obtuvo un rendimiento óptimo de 2,918 kg/ha con la aplicación 68 kg N/ha. La rotación del maíz endogámico con caupí no afectó ($P>0.05$) el rendimiento de la semilla. La altura de la planta, y los valores de SPAD-502® y de GreenSeeker® fueron indicadores adecuados de la suficiencia de N durante las etapas vegetativas V6 a V12, con valores óptimos de 149 cm, 46, y 0.67 NDVI, respectivamente, a los 52 días después de siembra con la aplicación de 68 kg N/ha. La respuesta del cultivo al fertilizante N ocurrió a un menor nivel que en estudios previos en sistemas de producción bajo condiciones comerciales. Otros factores relacionados con el manejo de fertilizante N tales como las fuentes de N, la ubicación y el momento de la aplicación deben ser considerados para lograr un sistema eficiente con buenos rendimientos de las semillas de maíz endogámico.

Palabras clave: líneas puras de maíz, coberturas, fertilizante nitrogenado, respuesta del cultivo

INTRODUCTION

Inbred maize occurs as a result of various generations of self-pollination to achieve homozygosity and is the genetic material for commercial hybrid seed production. Inbred maize lines differ from commercial hybrids in terms of yield, biomass, harvest index, nitrogen (N) use efficiency, and N uptake (Stringfield and Salter, 1934; Poehlman and Sleeper, 1995; Kiesselbach, 1999; Echarte and Tollenaar, 2006). Adequate agronomic management including adequate N fertilizer rates, sources, timing and placement are necessary to achieve high inbred maize yields.

Recent studies in Mollisols on the southern semi-arid coast of Puerto Rico have shown that optimum grain yields can be achieved with fertilizer N applications in the range of 84 to 112 kg N/ha (Sotomayor-Ramírez et al., 2012; Espinosa, 2015). However, it has been observed that some inbred maize producers may be applying relatively high levels of fertilizer N in efforts to improve seed yields (Sotomayor-Ramírez, unpublished data). Fertilizer N applications above crop requirements decrease the efficiency of fertilizer N use leading to potential losses by leaching, runoff, denitrification, and volatilization or by remaining as residual soil N. To maximize N use efficiency, N fertilizer management requires applying fertilizer N close to crop N requirement and synchronization between N availability and crop demand (Cassman et al., 2002). A soil may be poised to supply relatively high N levels, but plant N may be limited by the rate of supply in a given growth period as a result of soil N mineralization, thus the need for supplemental fertilization.

The availability of N to crops can consider N credits from previous rotation, cover crops, residual soil N, and soil N mineralization (Rice and Havlin, 1994). Previous studies indicate that maize in rotation with cover crops tends to have higher yields compared to monoculture (Tester and Landridge, 2010; Mupangwa et al., 2012; Sotomayor-Ramírez et al., 2012). This response is the result of the cover crop capacity for nutrient recycling, and its contribution to the formation of soil organic matter, which improves the availability of N to the successive crop (Peoples et al., 2009; Veras et al., 2016). Further adjustments to the N availability estimate can include N losses and immobilization (Cabrera et al., 1994). The difference between crop N requirement and available soil N can be provided by fertilizer N. Yet, such a large amount of information may be impractical to obtain, and, since not all this information is available a priori, fertilizer N recommendations are usually made based on crop N response and are related to crop N internal utilization. If crop response is not expected, fertilizer N application can be applied based on grain N export plus a correction factor to adjust for internal N requirements (Meisinger et al., 2008). The N management adjustments to maize also consider in-season indicators such as the pre-sidedress nitrate test (PSNT) (Magdoff, 1991), biomass N concentration (Ercoli et al., 1993), crop growth measurements (Sindelar et al., 2013) and the use of crop sensors to determine crop vigor (Tubaña et al., 2008; Sindelar et al., 2013).

The objective of this experiment was to evaluate the effect of six N fertilizer levels and N credits from the rotation with cowpea (*Vigna unguiculata*) on the yield and grain number of an inbred maize line produced by Dow Agrosciences[6]. In addition, we sought to determine

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

the accuracy of plant height measurements, chlorophyll readings using SPAD-502® and GreenSeeker®, and leaf N concentration as predictors of crop N sufficiency.

MATERIALS AND METHODS

The experiment was conducted at the Dow Agrosciences® facilities in Santa Isabel, Puerto Rico (17°58'37.5"N 66°24'03.9"W). The soil series was Jacaguas (Loamy-skeletal, mixed superactive, isohyperthermic Fluventic Haplustolls) (USDA-NRCS, 2000; USDA-NRCS 2016) of general high fertility, with a mean soil organic matter content of 20 g/kg and soil extractable NO_3^- of 31.7 kg N/ha (Table 1). The soil had been under maize monoculture for the past eight years and only in the previous two years had been rotated with a cover crop.

The experiment was conducted in an experimental field that had been established in the summer of 2011 by Espinosa (2015), who evaluated the effect of a cowpea (*Vigna unguiculata* cv. Iron clay) rotation and three levels of fertilizer N (60, 110 and 160 kg N/ha) on inbred maize yield. We overlaid the experiment within the same subplots as Espinosa (2015) and subdivided the three fertilizer N levels into two additional levels each to create our six fertilizer N levels. Thus, our experimental design was a randomized complete block in split-plot arrangement with four replications, with the main plots being the cowpea rotation and the six fertilizer N rates (0, 34, 68, 102, 135 and 203 kg N/ha) the subplots. Each of the experimental plots had a two-year antecedent cropping of cowpea or fallow and three fertilizer N levels. The main plot size was 83.6 m^2, and each subplot was 38.3 m^2.

The sources of fertilizer N were ammonium sulfate (21-0-0) and urea (46-0-0) at a 3:1 (NH_4^+-N:NO_3^--N) ratio. Fertilizer applications were made by irrigation at emergence or about six days after planting (DAP), 21 DAP and 37 DAP; the last two were supplemented by manual applications of granular fertilizer by banding in order to reach the desired N levels (Table 2). At planting, complementary nutrients (phosphorus, potassium and micronutrients) were applied at levels of 67 kg P_2O_5/ha, 112 kg K_2O/ha and 28 kg micronutrient-mix per hectare. The sources of P_2O_5, K_2O and essential minor elements were triple superphosphate (0-45-0), muriate of potash (0-0-60) and Five Star Mix ® (2.6% B, 4.8% Cu, 12.9% Fe, 9.4% Mn and 8.6% Zn), respectively.

Cowpea was grown prior to and after the maize cropping. The antecedent cowpea was planted on 13 July 2013, harvested on 20 September 2013 and incorporated into the soil shortly thereafter. A maize inbred line was selected with similar yields to the middle range of other lines planted throughout the farm. Maize was planted on 19 December 2013

Table 1.—*Soil fertility parameters*[1]*of field plots prior to maize planting at 0- to 15-cm depth for each of the rotation systems.*

Rotation system[2]	pH	SOM	Extractable-P	Ca	Mg	K	Na	CIC	Fe	Mn	Zn	Cu	B	SO_4-S
		g/kg	mg/kg	------------------cmol$_c$ /kg----------------					---------------------------mg/kg----------------					
F-M	7.65	21.0	36.2	17.3	5.2	0.67	0.39	23.4	17.5	2.5	1.7	5.5	0.4	4.8
C-M	7.65	19.0	29.1	19.4	5.1	0.49	0.34	25.3	17.1	1.3	1.2	4.9	0.4	3.8

[1] For pH measured on 1:1 soil:water, soil organic matter (SOM), extractable P, exchangeable cations (Ca, Mg, K, Na), cation exchange capacity (CIC), micronutrients (Fe, Mn, Zn, Cu, B), and SO_4-S. http://agsource.crinet.com/page298/Agronomy.

[2] F-M is the fertilizer maize rotation and C-M is the cowpea-maize rotation.

TABLE 2.—*Time of application and fertilizer N levels applied to treatments.*

	-------------------------Fertilizer N level (kg/ha) -------------------------					
Days after planting	0	34	68	102	135	203
At emergence	0	17 B[1]	17 F[2]	17 F+17 B	34 F	34 F+34 B
21	0	17 B	34 F	34 F+17 B	67 F	67 F+34 B
37	0	0	17 F	17 F	34 F	34 F

[1] Values with B indicate fertilizer was applied by banding (B) on the soil surface.

[2] Values with F indicate fertilizer was applied via fertigation (F).

and harvested on 19 March 2014. The planting rate was 51,645 plants per hectare at a row distance of 76 cm. Cowpea was again planted after the maize crop on 11 June 2014, harvested on 14 July 2014 and incorporated into the soil shortly thereafter. During the maize crop growth temperatures fluctuated between 21 and 30 °C. Growing degree-days ([Tmax + Tmin)/2) - 8° C]) to harvest was 1,640° Cd. Rainfall recorded during the crop-growth period (92 days) was 109 mm. Pest management and irrigation, provided by drip irrigation, followed established farm procedures.

Within each plot a randomly selected 1 m^2 quadrant was placed and the cowpea vegetative biomass was sampled at the ground level. Because there was strong weed pressure in 2013, cowpea and weeds were separated as independent samples. The fresh weight of the sample was measured. Plant biomass moisture was measured by weight loss of a 500 g subsample at 60° C, and total N concentration was analyzed.

After each cowpea incorporation, on 20 September 2013, and 11 July 2014, profile soil inorganic N was determined with a hand auger sampler at 0- to 15-, 15- to 30-, 30- to 60- and 60- to 100-cm-depth intervals in sub-plots with N levels of 0, 68 and 135 kg N/ha, and at 0- to 15- and 15- to 30-cm depth in subplots with N levels of 34, 102 and 203 kg N/ha. At 27 DAP, soil samples were taken to a depth of 0 to 30 cm in plots with N fertilizer levels of 0, 68 and 203 kg N/ha and analyzed for soil inorganic N.

During the maize growth period, agronomic parameters (plant height, indicator leaf N concentration, leaf greenness) were measured as treatment indicators and as predictors of crop yield. Plant height was measured on five randomly selected plants. Measurements were taken from the base of the stem to the last fully-extended leaf. Leaf greenness was measured with a SPAD-502® (Konica Minolta, Tokyo, Japan) and GreenSeeker® (NuTech Industries, Inc., Ukiah, CA) at 33, 42, 52 and 62 DAP. The SPAD readings were taken from ten plants in each plot, at the middle of the leaf laminae, using the last fully developed leaf (Mengel, 2008). GreenSeeker® values were recorded by placing the device at a distance of 80 to 120 cm in a vertical position over plant canopy (Barker and Sawyer, 2010) at a walking speed of 1 m/s over a 10-m distance (Teal et al., 2006; Tubaña et al., 2008). At 56 DAP the last fully developed leaf was collected in ten randomly selected plants and analyzed for N concentration. Grain and stover yields were determined at 91 DAP after randomly choosing three linear meters in each plot and collecting maize and plant biomass. Maize grain samples were dried in an industrial oven to 15.5% moisture. Fresh weight, dry weight and grain units were determined to calculate grain yields. Grain and stover samples were analyzed for N concentration.

Data Analysis

The data was analyzed as a mixed-model using the statistical package Infostat (2012) interfaced with R scripting language. An analysis of variance (ANOVA) was performed to test the effects of the main and subplot treatments on crop yield, plant biomass, SPAD, GreenSeeker, and plant height. The assumptions of normality and homogeneity of variance were verified with Shapiro-Wilks and Levene tests, respectively. The LSD Fisher test was performed to identify significant differences between treatments at $P<0.05$. A linear-plateau regression model was evaluated to test the effect of fertilizer N and available N (pre-plant soil N plus fertilizer N) vs. grain yield. Various fertilizer N use efficiency indicators were calculated using the partial productivity factor (PPF), biological productivity factor (BPP), agronomic efficiency (AE) and biological efficiency (BE). The partial productivity factor (PPF) was calculated as seed-yield/fertilizer N level applied; the biological productivity factor (BPP) was calculated as the seed-N uptake/fertilizer N level applied; the agronomic efficiency (AE) was calculated as (Seed $\text{yield}_{\text{N applied}}$-Grain $\text{yield}_{\text{N,0}}$)/fertilizer N level applied; the biological efficiency (BE) was calculated as biomass-N/ fertilizer N level applied (Cooke, 1987; Uhart and Andrade, 1995; Sotomayor-Ramírez et al., 2012).

RESULTS AND DISCUSSION

The rotation x fertilizer N level interaction was not significant for any of the variables evaluated ($P>0.05$; Table 3). Therefore, the results will be discussed in terms of the rotation and main effects of fertilizer N levels.

Cowpea cover crop biomass

Cowpea growth in summer 2013 had strong weed pressure. Residual N from the antecedent fertilization of maize planted in 2013 (Espinosa, 2015) did not affect ($P>0.05$) cowpea biomass, N concen-

TABLE 3.—*Summary of analysis of variance and significance levels for agronomic parameters collected days after planting (DAP) during the experiment. Grain yield was expressed at 15.5% humidity. The biomass was calculated as the sum of grain and stover.*

Agronomic variables		Rotation (R)	N Level (N)	R x N
Yield	Grain	0.1240	0.0020	0.2910
	Stover	0.0990	0.1320	0.3610
	Biomass	0.0100	0.0030	0.7400
Harvest index		0.0563	0.0053	0.2220
Grain units		0.0761	0.0212	0.4551
N harvest index		0.0590	0.0045	0.3067
N uptake	Grain	0.0250	0.0020	0.1850
	Stover	0.3930	0.0290	0.5330
	Biomass	0.0620	0.0010	0.4810
N leaf concentration		0.2420	0.2420	0.2430
Plant height	42 DAP	0.0040	0.0210	0.7890
	52 DAP	0.0670	0.0480	0.8750
SPAD-502	33 DAP	0.0110	0.0170	0.7370
	42 DAP	0.0820	0.0070	0.7900
	52 DAP	0.1170	0.0030	0.5810
	62 DAP	0.2510	0.1030	0.6800
GreenSeeker	33 DAP	<0.0001	0.0120	0.5380
	42 DAP	0.0030	0.0020	0.3110
	52 DAP	0.0040	0.0190	0.3890
	62 DAP	0.1390	0.0030	0.8080

tration and N uptake in summer 2013. The mean total aboveground biomass (dry weight) and N contribution was 2,593 kg/ha and 48 kg N/ha, respectively, with 44% of the biomass and 57% of the vegetative-N originating from the cowpea. The absence of N residual effect in cover-crop biomass differs from observations by Sultana et al. (2005) who reported that cowpea biomass increases with soil available N at the time of planting.

After summer 2013, cowpea was harvested and incorporated into the soil, maize was planted in winter 2014, followed by summer 2014 cowpea. Residual N from the antecedent fertilization of maize planted in 2014 did not affect cowpea biomass, N concentration and N uptake in summer 2014 (P> 0.05; Table 3). The cowpea dry-matter biomass production was 1,262 kg/ha, with N uptake of 48 kg/N ha.

Grain, stover and biomass yield

Maize grain yield was not affected by the rotation but was significantly affected by fertilizer N (P<0.02; Table 4). The absence of an increase in maize grain yield as a result of cowpea rotation contrasts with

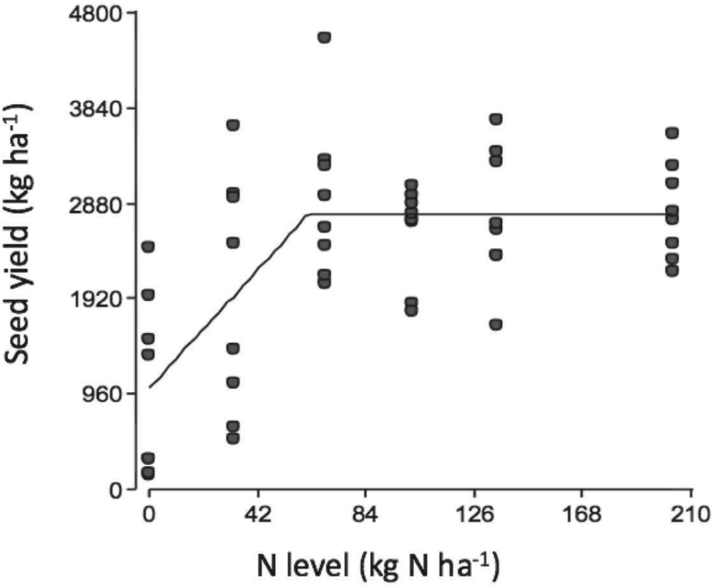
TABLE 4.—*Fertilizer N treatment effects on maize grain yield (15.5% moisture), stover, total biomass, harvest index, N harvest index, grain number, grain N, stover N, and biomass N.*

N level	Grain	Stover	Biomass	Harvest Index	N Harvest Index	Grain number	Grain N	Stover N	Biomass N
kg N/ha		kg/ha				$x10^6 ha^{-1}$		kg N/ha	
0	1,017 c[1]	5,179	5,915 c	0.11 c	0.16 c	2.45 b	10 b	49 c	59 c
34	1,951 b	5,693	7,051 b	0.18 b	0.23 bc	5.25 ab	19 b	61 ab	80 b
68	2,918 a	6,021	7,911 ab	0.24 ab	0.32 a	7.48 a	26 a	57 bc	83 b
102	2,588 a	6,299	8,228 a	0.23 ab	0.29 ab	8.36 a	26 a	64 ab	90 ab
135	2,754 a	5,810	7,721 ab	0.25 a	0.32 a	6.53 a	26 a	56 bc	82 b
203	2,798 a	6,310	8,434 a	0.25 a	0.31 ab	7.06 a	29 a	67 a	96 a
		NS[2]							

[1] Parameters with different letters in the same column are significantly different ($P<0.05$) as determined with LSD Fisher test.
[2] NS denotes non-significance ($P>0.05$) as determined using ANOVA.

reports by Tester and Landridge (2010), Mupangwa et al. (2012) and Sotomayor-Ramírez et al. (2012) who observed an increase in maize yield after cowpea incorporation before the maize planting. Maximum grain yield of 2,918 kg/ha was obtained with N fertilizer at 68 kg N/ha, which resulted in a 187% increase over the control. Fertilizer N between 68 and 203 kg N/ha resulted in yields between 2,588 and 2,918 kg/ha, with no significant differences among yields. Previous studies reported lower grain yields under non N fertilizer application (Tubaña et al., 2008; Szulc, 2013). The linear-plateau model showed an optimum N level of 62 kg N/ha with a predicted grain yield of 2,770 kg/ha (Figure 1). The optimum fertilizer N values coincide with results by Espinosa et al. (2015) who found no significant differences in inbred maize grain yield with N fertilizer levels between 60 and 160 kg/ha.

Stover yield was not affected by rotation, fertilizer N level or by their interaction ($P>0.05$). Maize biomass was 10% higher under fallow as compared to the cowpea rotation with values of 7,972 and 7,115 kg/ha, respectively ($P<0.05$) and the optimum fertilizer N level was 68 kg N/ha



$y=1,016+28(N)$; when $N<62$; $y=2,770$; when $N\geq62$

FIGURE 1. Linear-plateau relationship between level of N and crop yields model, *N* and *y* term in the equation, respectively.

($P<0.05$) (Table 4). Sotomayor-Ramírez et al. (2012) reported that stover yields were affected by fertilizer N in one of the two years evaluated.

The highest harvest index (HI) was 0.24 with the N fertilizer level of 68 kg N/ha (Table 4). Results coincide with Sotomayor-Ramírez et al. (2012) who reported inbred HI of 0.25 to 0.27 with N fertilizer levels between 0 and 112 kg N/ha. There was no significant increase in grain number after 34 kg N/ha; with 114% more grains compared to the control treatment (0 kg N/ha). The N harvest index (NHI) increased 100% with 68 kg N/ha compared with the control treatment (0 kg N/ha), and NHI did not increase thereafter. In summary, optimum HI, NHI and grain number were attained with fertilizer N levels of 68, 68 and 34 kg N/ha, respectively. Addition of fertilizer N significantly increased grain, stover and biomass N uptake with maximum values for 68, 34 and 102 kg N/ha, respectively.

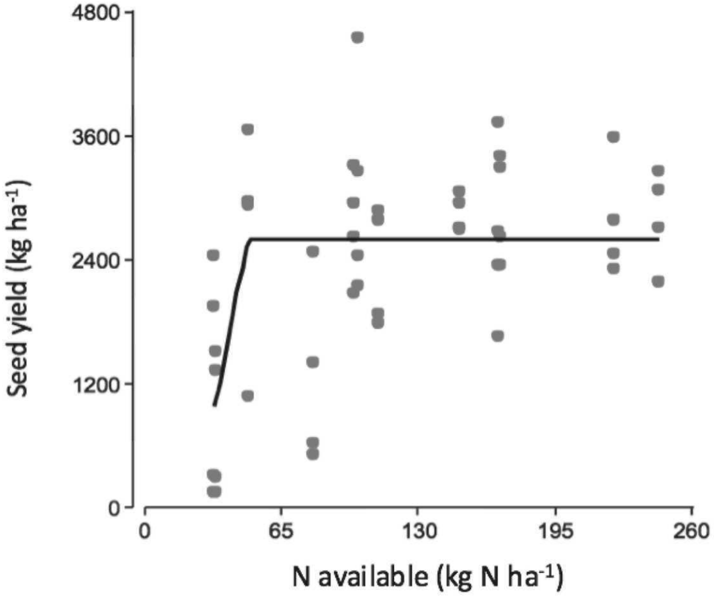
A priori agronomic indicators of yield

Maize pre-plant inorganic N availability in soil profiles (0 to 90 cm) ranged from 66.7 to 77.4 kg/ha (Table 5). Pre-plant immediately available inorganic N ranged from 15.9 to 47.1 kg N/ha, and potentially leachable inorganic-N (30 to 90 cm) was 15 to 25% higher than profile available N (0 to 30 cm). Amado and Mielniczuk (2000), and Satorre et al. (2001) recommend that both pre-plant soil N availability and fertilizer N be considered to satisfy crop N demand. Thus, we used the sum of pre-plant soil N at 0 to 30 cm and fertilizer N applied as available N. We fit the experimental yield data to available N with a linear-plateau model and determined that the optimal available N level was 49 kg N/ha with a grain yield of 2,600 kg/ha (Figure 2), which is 13 kg N/ha less than just considering fertilizer N fertilizer applied (62 kg N/ha).

The rotation (Table 6) and fertilizer N levels (Table 7) had a significant effect on agronomic predictors plant height, SPAD-502 and

TABLE 5.—*Pre-plant total inorganic N availability for soil samples collected in fall 2013 in the fallow-maize (F-M) and cowpea-maize (C-M) rotation.*

	--------------------F-M--------------------			--------------------C-M--------------------		
N level	0-30 cm	30-90 cm	0-90 cm	0-30 cm	30-90 cm	0-90 cm
kg/ha	--kg/ha--					
0	33.3	38.4	71.7	34.2	41.8	76.0
34	15.9			47.1		
68	31.3	35.3	66.7	33.2	44.2	77.4
102	47.6			9.2		
135	34.2	42.7	76.9	33.1	42.5	75.5
203	42.0			20.3		



$y=-2,504+104(N)$; when $N<49$s; $y=2,600$; when $N\geq 49$

FIGURE 2. Relation between N available and grain yields using a linear-plateau model, *N* and *y* term in the equation, respectively.

GreenSeeker; this varied according to the vegetative growth stage ($P<0.05$). The rotation showed significant differences in plant height at 42 DAP ($P<0.05$) with values of 120 cm and 108 cm for fallow-maize (FM) and cowpea-maize (CM), respectively. The SPAD-502 index was affected by the rotation at 33 DAP with values of 40 and 37 for FM and CM, respectively ($P<0.05$).

Sotomayor-Ramírez et al. (2012) reported that plant height and SPAD-502 index of inbred maize lines were influenced by fertilizer N levels applied ($P<0.05$). However, we found no significant differences in plant height with N fertilizer application greater than 68 kg N/ha, with values of 113 and 149 cm for 42 and 52 DAP, respectively. The SPAD-502 values at 33, 42, 52 and 62 DAP were 39, 46, 43 and 47, respectively. Sotomayor-Ramírez et al. (2012) reported SPAD-502 values of 50, 53 and 54 for 40, 54 and 70 DAP, when monitoring an inbred maize line with N fertilizer levels in the range of 112 to 224 kg N/ha.

GreenSeeker readings were significantly affected by the FM and CM rotations at 33, 42 and 52 DAP ($P<0.05$) with values of 0.60

TABLE 6.—*Effect of the cover crop rotation fallow-maize (FM) and cowpea-maize (CM) on agronomic parameters plant height, SPAD-502 and GreenSeeker readings at 33, 42, 52 and 62 days after planting (DAP).*

		Rotation	
Agronomic parameters	DAP	FM	CM
Plant height (cm)	42	120	108*[1]
	52	151	143
SPAD-502	33	40	37*
	42	46	44
	52	42	41
	62	48	46
GreenSeeker	33	0.60	0.51*
	42	0.66	0.60*
	52	0.67	0.63*
	62	0.59	0.58

[1] Means with * in the same row, for individual agronomic parameters, are significantly different as affected by rotation as determined with LSD Fisher test.

and 0.51, 0.66 and 0.60, and 0.67 and 0.63, respectively. GreenSeeker highest values at 33, 42, 52 and 62 DAP were achieved with N fertilizer levels of 34, 102, 68 and 34, respectively. GreenSeeker NDVI values corresponding to the 33, 42, 52 and 62 DAP were 0.55, 0.64, 0.67 and 0.59, respectively. Teal et al. (2006) and Tubaña et al. (2008) have previously reported a positive relationship between GreenSeeker values and crop N availability during the V6 to V9 stages.

The N concentration of the indicator leaf at 56 DAP showed a significant correlation grain yield (Figure 3), with an optimal N concentration of 2.5% for a grain yield of 2,660 kg/ha. Sotomayor-Ramírez et al. (2012) reported values from 2.14 to 3.31% N as optimun N concentration levels for the indicator leaf measured on maize inbred lines.

A posteriori agronomic indicators

After the 2014 summer cover crop, the residual soil profile N (0 to 90 cm) was quantified in plots with a history of fertilizer N application of 0, 68 and 203 kg N/ha. Rotation and fertilizer N levels showed no differences in the amount of inorganic N in soil profile (Table 8). An average of 48.5 kg N/ha in the soil profile (0 to 90 cm) was the contribution presumed by the mineralization of N, fixing atmospheric N and N in irrigation water and precipitation (Cabrera, 2007; Celaya and Castellanos, 2011). The amount of immediately available soil N (0 to 30 cm) was about 50% higher compared to the potentially leached N (30 to 90 cm).

TABLE 7.—*Effect of N levels on agronomic parameters plant height, SPAD-502 and GreenSeeker at 33, 42, 52 and 62 days after planting (DAP).*

		Fertilizer N level (kg/ha)					
Agronomic parameters	DAP	0	34	68	102	135	203
Plant height	42	103 b[1]	105 b	113 ab*	121 a	121 a	122 a
	52	135 c	140 bc	149 ab*	152 ab	153 a	155 a
SPAD-502	33	35 c	37 bc	39 ab*	41 a	39 ab	41 a
	42	40 c	43 bc	46 ab*	47 a	47 a	47 a
	52	38 c	40 bc	41 bc	40 bc	43 ab*	47 a
	62	41 b	47 ab*	46 ab	48 ab	51 a	48 ab
GreenSeeker	33	0.49 b	0.55 a*	0.57 a	0.57 a	0.56 a	0.57 a
	42	0.55 c	0.60 b	0.61 b	0.64 ab*	0.69 a	0.68 a
	52	0.61 b	0.63 b	0.67 a*	0.67 a	0.66 a	0.66 a
	62	0.55 b	0.59 a*	0.60 a	0.60 a	0.60 a	0.60 a

[1] Parameters with different letters in the same row are significantly different ($P<0.05$) as determined with LSD Fisher test.

*There were no significant differences after the specific N level for the parameter.

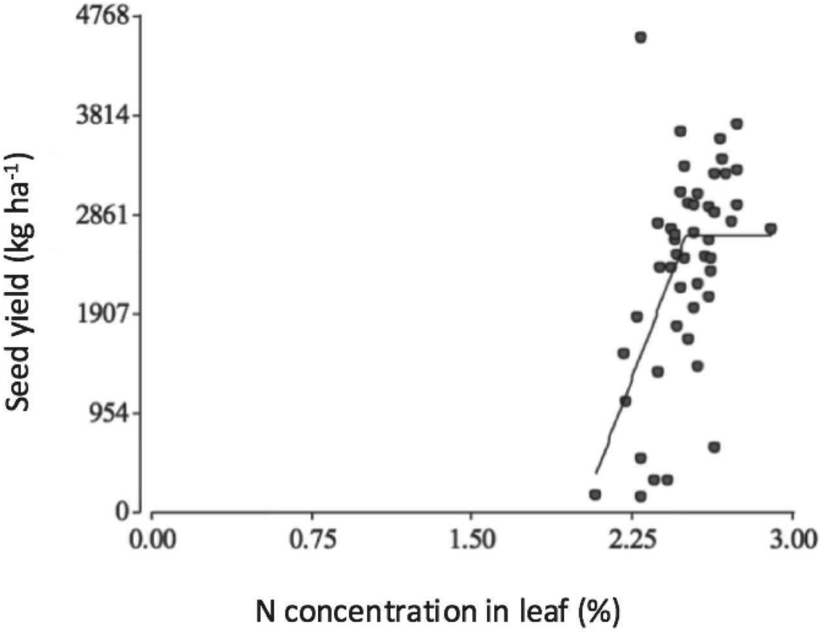


FIGURE 3. Linear-plateau relationship between N concentration in leaf (N) and crop yields (y) model.

N fertilizer use efficiency

In general, N use efficiency showed a decreasing trend with the increase of fertilizer N levels (Table 9). The optimal values of PPF were 59.1 and 42.9 with N levels of 34 and 68 kg N/ha, respectively. Snyder and Bruulsema (2007) reported optimum values for PPF between 40 and 80 for cereal crops. Values for production of maize inbred lines in the southern part of Puerto Rico have been reported at 17.5 and 24.4 PPF with 84 and 112 kg N/ha, respectively (Sotomayor-Ramírez et al., 2012). The BPP decreased with applications greater than 34 kg N/ha with a value of 0.58. Sotomayor-Ramírez et al. (2012) reported values of 0.35 and 0.21 BPP with N fertilizer levels of 84 and 112 kg N/ha, respectively. There was no significant increase in AE with applications between 34 to 135 kg N/ha. The AE with 34 kg N/ ha was 28.3, which is in the range of optimal values from 10 to 30 for AE reported by Snyder and Bruulsema (2007). The BE decreased significantly with applications greater than 34 kg N/ha. According to the PPF, the BPP, the AE and BE, N greater efficiency in the production of the inbred maize line evaluated in this study is 34 kg N/ha.

TABLE 8.—*Total inorganic N soil availability after rotation during summer 2014.*

	Fallow-Maize			Cowpea-Maize		
N Level	0-30 (cm)	30-90 (cm)	0-90 (cm)	0-30 (cm)	30-90 (cm)	0-90 (cm)
kg N/ha	kg N/ha					
0	30	16	46	32	17	49
68	29	13	42	36	17	53
203	34	14	49	33	19	52

TABLE 9.—*Fertilizer N use efficiency indicators for inbred maize. The fertilizer N levels and partial productivity factor (PPF), biological productivity factor (PPB), agronomic efficiency (AE), and biological efficiency (BE).*

N Level kg N/ha	PPF	BPP	AE	BE
0	—	—	—	—
34	59.1 a[1]	0.58 a	28.3 a	2.42 a
68	42.9 ab	0.39 b	27.9 a	1.22 b
102	25.4 bc	0.26 bc	15.4 ab	0.89 c
135	20.4 c	0.20 c	12.8 ab	0.61 d
203	13.8 c	0.15 c	8.77 b	0.47 d

[1] Means with different letters within columns are significantly different as determined with LSD Fisher test.

CONCLUSIONS

The use of cowpea during fallow prior to maize planting did not significantly affect maize grain yield. There was a positive quantitative relation between fertilizer N level applied, crop growth and grain yield. Highest yield reported in this study was 2,918 kg/ha with an optimum rate of 68 kg N/ha ($P<0.05$). Considering soil N availability (inorganic N in the soil at a depth of 0 to 30 cm + N applied) the optimal modeled yield was 2,600 kg/ha with a N fertilizer availability of 49 kg N/ha. Nitrogen concentration in leaf, grain, and biomass at 56 DAP at harvest are appropriate indicators of crop N sufficiency. Plant height, SPAD-502 and GreenSeeker readings are suitable indicators of crop N sufficiency. Nitrogen fertilizer dose recommendations can be refined considering the crop growth stage and these agronomic indicators for crop vigor.

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