Agronomic parameters as indicators of inbred maize nitrogen status[1,2]

Ada Vilches-Ortega[3], David Sotomayor-Ramírez[4], Johanis Rivera-Zayas[5] and Ricardo Barnes[6]*

J. Agric. Univ. P.R. 106(1):33-46 (2022)

ABSTRACT

Inbred maize (*Zea mays* L.) lines are grown for seed in Puerto Rico. In efforts to increase crop yields, some producers may apply nitrogen (N) fertilizer in excess of crop nutrient requirements. The use of cover crops (CC) in rotation can serve as an alternative to continuous cultivation of maize. This experiment was conducted in field plots of Güamaní (fine-loamy over sandy or sandy-skeletal, mixed, superactive, isohyperthermic Torrifluventic Haplustepts) soil in Guayama, Puerto Rico. The cropping systems evaluated were a maize-cowpea [*Vigna unguiculata* (L.) Walp.] cover crop rotation versus a typical practice of maize fallow (FA), each with five N fertilizer levels. The maize planting sequence was an initial spring 2014 planting, followed by a winter 2014-2015 planting and a final winter 2015-2016 planting. The cowpea cover crop was planted in the summers of 2014, 2015 and 2016. The inbred maize lines were female lines used for commercial hybrid maize production. Mean maize plant densities ranged from 59,391 to 69,182 plants per hectare for the cropping seasons. Indicators of crop N status were the Soil Plant Analysis Development (SPAD-502) Chlorophyll Meter®measuring leaf greenness; the Normalized Difference Vegetation Index (NDVI, measured with GreenSeeker®) and plant height measurements. The application of N fertilizer significantly influenced ($p<0.05$) leaf greenness (in five out of 13 occasions) and plant height (in seven of 12 occasions) but not NDVI. The use of cover crop significantly influenced leaf greenness (in four out of nine occasions) and plant height (in three out of eight occasions). The agronomic parameters tested did not predict yield or crop N status, thus are not recommended in the decision-making process of N fertilizer management of inbred maize.

Key words: inbred maize, fertilizer nitrogen, crop nitrogen status

[1]Manuscript submitted to Editorial Board 30 September 2021.

[2]We acknowledge the cooperation of the Dow-AgroSciences Staff and UPRM-AES graduate and undergraduate students. Funding for this study was provided by Dow AgroSciences, LLC.

[3]Former graduate student, University of Puerto Rico-Mayagüez Campus; currently at USDA-NRCS, Bangor, ME.

[4]Professor, Department of Agroenvironmental Sciences, College of Agricultural Sciences, University of Puerto Rico – Mayagüez Campus, PO Box 9030, Mayagüez, Puerto Rico; *Corresponding author email: david.sotomayor@upr.edu.

[5]Former graduate student, University of Puerto Rico-Mayagüez Campus; currently at University of Hawaii.

[6]Former Field Station Operation Leader, Dow AgroSciences, LLC, Santa Isabel, PR.

RESUMEN

Parámetros agronómicos como indicadores del estatus de nitrógeno en líneas puras de maíz

En Puerto Rico se cultivan líneas de maíz (*Zea mays* L.) endogámicas para semilla. En aras de lograr mayores rendimientos, algunos productores aplican niveles altos de fertilizante nitrogenado (N). El uso de plantas cobertoras (CC) en rotación puede servir como alternativa a un sistema de maíz continuo. Este experimento se realizó en un suelo Güamaní (*fine-loamy over sandy or sandy-skeletal, mixed, superactive, isohyperthermic Torrifluventic Haplustepts*) en el municipio de Guayama. Los sistemas de cultivo evaluados fueron un monocultivo de maíz versus una rotación de cultivo de maíz-caupí (*Vigna unguiculata* (L.) Walp.), con cinco niveles de fertilizante nitrogenado. La secuencia de maíz fue una siembra inicial en la primavera del 2014, después en el invierno 2014-2015 y la última siembra fue en el invierno del 2015-2016. La cobertora se estableció en los veranos del 2014, 2015 y 2016. Las líneas de maíz cultivadas fueron líneas hembras, las cuales se utilizan en la producción comercial de maíz híbrido. La densidad de plantas varió entre 59,391 y 69,182 plantas por hectárea para los años de estudio. El verdor de las hojas, medido con el *SPAD-502 chlorophyll meter*®, el índice de vegetación de diferencia normalizada (NDVI, por sus siglas en inglés), medido con *GreenSeeker*®, y la altura de la planta se utilizaron como indicadores del estatus de N en el cultivo. Las aplicaciones de fertilizante nitrogenado influyeron significativamente ($p < 0.05$) en el verdor de las hojas (en cinco de 13 ocasiones) y la altura de la planta (en siete de 12 ocasiones), pero no en el NDVI. Los indicadores agronómicos evaluados no predijeron adecuadamente el rendimiento ni la utilización de N, por lo que no se recomiendan en el proceso de toma de decisiones en el manejo de N en líneas puras de maíz.

Palabras claves: líneas puras de maíz, fertilizante nitrogenado, estatus de nitrógeno en cultivos

INTRODUCTION

The increase in world population coupled with heightened environmental consciousness prompted the need to ensure global food security using sustainable farming systems (Garibaldi et al., 2017). Maize (*Zea mays* L.) is the largest cereal grain crop globally in terms of quantity (FAOSTAT, 2018). Maize is used in a wide range of products to feed humans and livestock, to produce biofuel and for industrial uses (Ranum et al., 2014).

A major concern in maize cropping systems is appropriate nutrient management, especially nitrogen (N) fertilization. This concern arises from the difficulty of being able to adequately predict soil N availability to plants and crop N status. Nitrogen is an essential nutrient and usually the most limiting, because of large plant demands and ecosystem losses (Kant et al., 2011). Crops may use 30 to 60% of the N fertilizer applied because of the multiple N transformation processes encompassed in the ecosystem and because ammonium and nitrate are mobile compounds in the soil (Raun and Johnson, 1999). In the case of urea fertilizers, losses of N are generally larger with increasing soil pH, temperature and surface residue (Hargrove et al., 1961). Therefore, there is a tendency for producers to apply N fertilizer rates exceeding crop N requirements (Ju et al., 2009).

Over the last three decades, several companies have established operations in Puerto Rico focusing on the genetic improvement and production of hybrid maize seeds. Maize hybrids are produced from inbred lines to produce higher seed yields with greater tolerance to disease, pests and drought (Poehlman and Sleper, 1995). Inbred maize lines have lower seed yield potential and, in efforts to improve yields producers may apply N in excess of crop requirements (Sotomayor-Ramírez et al., 2012). One of the most important practices in inbred maize cropping systems is nutrient management, especially N fertilization. To establish an adequate nutrient management plan, predictors and analysis of crop N status should be used. Agronomic indicators such as reflectance sensors and chlorophyll meters can help to predict crop N status (Scharf, 2001; Muñoz-Huerta et al., 2013). Plant tissue and soil N analysis can also be used to determine N use efficiency and potential losses in the system (Meisinger et al., 2008). The objectives of this experiment were to evaluate if selected agronomic parameters were sensitive to N fertilizer and cover crop treatments and to evaluate these as predictors of crop yield and crop N status.

MATERIALS AND METHODS

The experiment was conducted in the municipality of Guayama, Puerto Rico, near latitude 17.968148 and longitude -66.184293, at a farm owned by a seed company. The company produced maize and soybean [*Glycine max* (L.) Merr.] seeds, and their maize growing season was from September to April. The soil where the experiment was located is Güamaní (fine-loamy over sandy or sandy-skeletal, mixed, superactive, isohyperthermic Torrifluventic Haplustepts).

The experiment was conducted over three years. The field site was under grassland before it was tilled and planted with maize. Cumulative precipitation during maize growth period was 157, 173 and 196 millimeters for the 2014, 2014-2015 and 2015-2016 seasons, respectively (Figure 1). Cumulative precipitation during the cover crop growth was 551, 376 and 274 millimeters for the 2014, 2014-2015 and 2015-2016 seasons, respectively (Figure 1). Supplemental irrigation to the maize cropping was provided by drip irrigation according to the company's established practice.

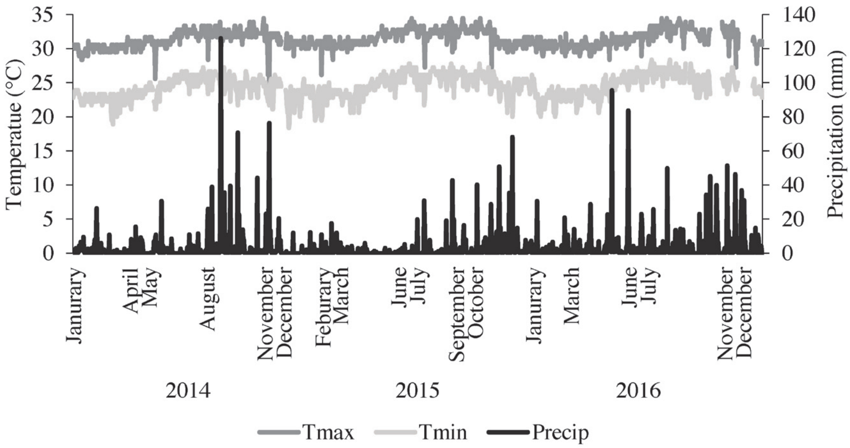

FIGURE 1. Precipitation (mm), maximum temperature (Tmax) and minimum temperature (Tmin) for experiment years. Weather date acquired from www.ncdc.noaa.gov/cdo-web/datatools/findstation. The weather station referenced was Guayama 2 E, US. Latitude: 17.9783. Longitude: -66.0874. Elevation: 23 m (72 ft).

The experimental design for the 2014 season was a randomized complete block design with four replicates. There was no cover crop rotation at this time. There were 20 main plots measuring 9 x 37 m, and each of these plots contained 12 rows of planted maize. Maize was planted on 14 April 2014 and harvested 16 July 2014. After this, the first cover crop rotation was established. The cover crop was planted on 8 August 2014 and incorporated 24 October 2014. For the 2014-2015 and 2015-2016 seasons, the design was converted to a strip-plot arrangement of a randomized complete block design with four replicates. The main plots were the five N fertilizer levels, and the strip was the cowpea [*Vigna unguiculata* (L.) Walp.] cover crop rotation. The main plots were 9 x 37 m and the sub-plots were 9 x 18 m. Each plot had 12 rows of maize when planted. For the 2014-2015 season, maize was planted on 10 December 2014 and harvested 23 April 2015. The cover crop was planted on 21 May 2015 and overseeded on 9 June 2015 due to poor germination. Cowpea was incorporated into the soil on 2 November 2015. Maize was planted on 21 December 2015, harvested 21 March 2016 and cowpea cover crop was planted on 15 June 2016 and incorporated 8 September 2016 for the 2015-2016 trial period. The inbred maize lines were female lines for commercial hybrid maize production. Both are tolerant to insects of the order Lepidoptera and resistant to glyphosate.

The five N fertilizer levels for both the 2014 and 2014-2015 seasons were: 0, 90, 135, 180 and 225 kg N/ha. The five N fertilizer levels for 2015-2016 were: 0, 50, 100, 150 and 200 kg N/ha. The fertilizer N sources were ammonium sulfate and urea at an urea-N:NH_4-N ratio of 3:1 (2014 and 2014-2015) and 1:1 (2015-2016). The N fertilizer applications were split, one at planting and one several days after planting (DAP) (Table 1). Side-dressing or placement of fertilizer between rows was by hand. Nitrogen was not applied to the control treatments (0 kg N/ha). At planting for all three seasons, all treatments received an application of 56, 93 and 25 kg/ha of P_2O_5, K_2O and ME (minor elements), respectively, applied in the form of triple superphosphate, potassium chloride and Granusol Five-Star-Mix®[7] to ensure that the only limiting nutrient for the maize was N. During maize growth, drip irrigation was provided twice a week depending on climate conditions. Scouting and pest control were done by company employees and applications were conducted following company best practices. Seeds of the cowpea variety 'Iron clay' were obtained from Johnny Seeds® for all years of the experiment. The cover crop was sprayed with glyphosate and incorporated during flowering (between 60 to 90 DAP). Afterwards, the field was tilled weekly until the next maize planting. No weed or pest control was used during cowpea growth.

Field Measurements

The SPAD-502 (Konica Minolta, Tokyo, Japan) was used to determine leaf greenness which is strongly related to leaf chlorophyll and

TABLE 1.—*Nitrogen fertilizer rates applied to inbred maize from 2014-2016.*

	N fertilizer applications[1]					
	2014		2014-2015		2015-2016	
N-level[2]	1st[3]	2nd[4]	1st	2nd	1st	2nd
	---------------------------kgN/ha---------------------------					
N2	68	22	68	22	30	20
N3	68	67	68	67	30	70
N4	68	112	68	112	30	120
N5	68	157	68	157	30	170

[1]N fertilizer applications were split in two.

[2]2014 and 2014-2015 total N fertilizer levels were 90, 135, 180 and 225 kg N/ha for N2, N3, N4 and N5, respectively. 2015-2016 N2, N3, N4 and N5 total N fertilizer levels were 50, 100, 150 and 200 kg N/ha, respectively.

[3]The first N fertilizer application was at planting.

[4]The second application was 36 DAP, 34 DAP and 38 DAP for the 2014, 2014-2015 and 2015-2016 seasons, respectively.

[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 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.

N concentration. Twenty plants were selected and measured at V6, V8, V10 and R1 stages for the 2014 season and at V6, V8, V10, R1 and R2 stages for the 2014-2015 and 2015-2016 seasons. The SPAD-502 measurement was taken from the youngest leaf with a fully expanded collar. As described by Mengel (2008), the measurement was made halfway between the leaf margin and the midrib and halfway between the leaf tip and the leaf collar every time.

Agronomic parameters

The GreenSeeker handheld crop sensor (NuTech Industries, Inc., Ukiah, CA) was used to determine plant greenness which is an indirect indicator of plant health and N sufficiency. The measurement was taken while walking parallel to the plants and at a height of 80 cm to 120 cm (31 to 47 inches) above the crop canopy in a 12 m (40 ft) segment. Measurements were made 3 m (10 ft) in from the border of the plots and at a pace of 1 m/s. The GreenSeeker provides data through its sensor that emits red and infrared light which are quantified as normalized difference vegetation index (NDVI) values that range from 0.00 to 0.99. These values are unitless (Mengel, 2008). Greenseeker measurements were taken the same days as SPAD-502 measurements for all years. Plant height was also measured on the same days. Ten plants were measured in one row of each subplot for the 2014-2015 and 2015-2016 seasons and in one row of each main plot for the 2014 season. The measurements were taken from the base of the trunk to the top of the plant while fully extending the leaves.

Data analysis

Statistical analysis was done using the software InfoStat (2014®). An ANOVA with a randomized complete block design arrangement was done for all agronomic indicator 2014 data. This ANOVA consisted of two factors: nitrogen level (N level) and replicate. The agronomic indicator data for the periods of 2014-2015 and 2015-2016 had a strip-plot in a randomized complete block design ANOVA arrangement with the factors of N level, rotation and replicate. A Shapiro-Wilks test was used to verify data normalization, and Levene for data homogeneity. Significant differences in the ANOVA analysis were determined using the means separation LSD Fisher test with an α of 0.05. Graphical plots and linear correlation analysis among the agronomic parameters measured and crop yield and crop N uptake could be used to evaluate these as predictors of crop yield and crop N status.

RESULTS AND DISCUSSION

Crop response to N fertilizer was achieved in two of three cropping sequences. Optimum yields were achieved with N fertilizer rate of 90 kg/ha for both the 2014 and 2014-2015 seasons (Vilches-Ortega et al., 2022). The relative yield increase to fertilization was 12.2% for 2014 and 36.6% in 2014-2015.

All three agronomic indicators were not significantly different ($p>0.05$) for the interaction of N fertilizer level x cover crop rotation for the two seasons (Table 2). Therefore, only the results pertaining to the principal effects of rotation or N fertilizer level will be discussed.

In the 2014 season, N fertilizer significantly affected plant height at V6 to V10 ($p<0.05$) but not at R1 stage (Table 3). Plant height has been previously observed to be affected by N treatments (Sotomayor-Ramírez et al., 2012; Muharam et al., 2014; Rivera-Zayas et al., 2017). Maximum plant height was achieved with N fertilizer level of 90 kg/ha, with no significant difference at increasing N fertilizer levels during V6 to V10 stages. The N fertilizer levels did not affect SPAD-502 or NDVI. The means for SPAD-502 measurements for all N fertilizer levels were 43.7, 43.7, 48.9 and 51.6 and means for NDVI were 0.66, 0.69, 0.74 and 0.74 at V6, V8, V10 and R1 stages, respectively. Rivera-Zayas et al. (2017) reported no significant differences in plant height with N fertilizer application greater than 68 kg N/ha.

In the 2014-2015 season, plant height and SPAD-502 were influenced by N fertilizer levels at varying growth stages (Table 4). The NDVI measurements were not influenced by the level of N treatment. Mean NDVI values averaged across N fertilizer levels were 0.61, 0.72, 0.73, 0.73 and 0.69 for V6, V8, V10, R1 and R3 stages, respectively. Differences in plant heights at 45, 52 and 59 DAP were not significant for N fertilizer rates greater than 135 kg N/ha. Sotomayor-Ramírez et al. (2012) and Rivera-Zayas et al. (2017) also reported significant differences in plant height and SPAD-502 measurements for endogamic maize lines in Puerto Rico. The SPAD-502 values for almost all N levels increased as the season progressed. At optimum fertilizer levels, Sotomayor-Ramírez et al. (2012) reported SPAD-502 values ranged from 51 to 53 at 40 to 54 DAP, respectively, and Rivera-Zayas et al. (2017) reported SPAD-502 values of 39, 46, 43 and 47 at 33, 42, 52 and 62 DAP, respectively. The chlorophyll concentration as measured by SPAD-502 demonstrates the importance of developing line-specific SPAD-502 optimum values.

In the 2015-2016 season, plant height was significantly higher at N fertilizer rate of 50 kg N/ha at the V8 stage (Table 5) with a value

TABLE 2.—*Summarized ANOVA (p-values) for the effects of N fertilizer and cover crop rotation on agronomic indicators of inbred maize from 2014 to 2016. The fertilizer x cover crop interaction was not significant, and the ANOVA summary for the interaction was not included.*

	2014	2014-2015		2015-2016	
Agronomic Indicator	Fertilizer N	Fertilizer N	Cover crop	Fertilizer N	Cover crop
Plant Height 1[1]	0.0145	0.0735	0.2030	0.0094	0.0051
Plant Height 2	0.0043	0.0233	0.7417	0.0910	0.0017
Plant Height 3	0.0224	0.0036	0.1988	0.2256	0.0250
Plant Height 4	0.1199	0.0047	0.1932	0.8812	0.2055
NDVI 1[1]	0.8774	0.0668	0.2227	0.5539	0.4385
NDVI 2	0.4508	0.7171	0.4638	0.0633	0.0310
NDVI 3	0.2123	0.8609	0.0177	0.9146	0.7508
NDVI 4	0.5102	0.8342	0.0139	0.0934	0.9054
NDVI 5	--------[2]	0.9202	0.0565	--------[2]	--------[2]
SPAD 1[1]	0.5854	0.0359	0.7237	0.0454	0.0455
SPAD 2	0.1537	0.1765	0.0006	0.0957	0.0061
SPAD 3	0.0901	0.0324	0.1410	0.8311	0.0307
SPAD 4	0.6752	0.0323	0.1419	0.0945	0.2609
SPAD 5	--------[2]	0.0015	0.1533	--------[2]	--------[2]

[1]In 2014 cropping, plant height, SPAD and NDVI (GreenSeeker) measurements were taken at 29, 37, 44 and 49 DAP. In 2014-2015 plant height was taken at 31, 38, 45 and 52 DAP, while SPAD and NDVI were measured at 31, 38, 45, 52 and 59 DAP. Plant height, SPAD and NDVI for the 2015-2016 season were taken at 35, 42, 49 and 56 DAP.

[2]NDVI and SPAD data were not collected at a fifth date for the 2014 and 2015-2016 seasons.

TABLE 3.—*Effects of N fertilizer on plant agronomic indicators of inbred maize in 2014 cropping.*

Agronomic indicator	Growth stage[1]	Fertilizer Treatment (2014) 0	90	135	180	225	Means
		----------kg/ha----------					
Plant height (cm)	V6	79.3 a[2]	93.9 b*[4]	93.9 b	94.5 b	92.6 b	--------[5]
	V8	112.1 a	125.6 b*	128.1 b	128.4 b	126.3 b	--------
	V10	146.4 a	159.9 b*	162.8 b	162.3 b	159.1 b	--------
	R1	164.7 ns[3]	178.5	178.7	178.6	174.6	175.0
SPAD-502	V6	42.4 ns	44.9	43.7	43.5	44.0	43.7
	V8	43.2 ns	44.2	42.8	42.2	46.1	43.7
	V10	46.6 ns	49.2	54.5	47.2	46.9	48.9
	R1	49.5 ns	51.9	57.5	48.2	50.8	51.6
NDVI (GreenSeeker)	V6	0.66 ns	0.67	0.67	0.67	0.63	0.66
	V8	0.68 ns	0.69	0.70	0.70	0.70	0.69
	V10	0.75 ns	0.73	0.73	0.74	0.74	0.74
	R1	0.74 ns	0.72	0.75	0.74	0.75	0.74

[1]V6, V8, V10 and R1, correspond to 29, 37, 44 and 49 DAP, respectively.
[2]Means for each agronomic indicator with different letters within a DAP are significantly different as determined with Fisher's LSD test ($p<0.05$).
[3]ns denotes treatments were not significant at $p>0.05$.
[4]*No significant differences from this value onwards.
[5]Means were not calculated for significantly different data ($p<0.05$).

of 119.3 cm. The effect of N fertilizer at other growth stages was not significant. The SPAD-502 value was significantly higher at N fertilizer level of 50 kg N/ha at V8 stage with similar values at higher fertilizer rates. In contrast to the 2014 season and other studies, SPAD-502 values decreased as the season progressed. Tajul et al. (2013) also observed a decrease in SPAD-502 values with increasing plant age. All NDVI values were not significantly different and had means of 0.68, 0.73, 0.71 and 0.61 for 35, 42, 49 and 56 DAP, respectively.

The NDVI was not affected by N fertilizer treatments during the three seasons. This could be attributed to the GreenSeeker's sensitivity to NDVI values close to saturation levels. Tremblay et al. (2009) concluded that the GreenSeeker handheld sensor can be used to estimate crop N requirements before the V5 (Vn=maize vegetative stage (V) with n^{th} leaf collar visible) growth stage. In another study by Shaver et al. (2011) the GreenSeeker was able to determine N variability at the V12 and V14 growth stages. They also reported that the GreenSeeker could reach saturation levels earlier in the maize growing season. The authors suggest not using the GreenSeeker at later crop growth stages due to greater plant biomass. The GreenSeeker data in our study may be in accord with observations by Tremblay et al. (2009) who reported

TABLE 4.—*Effects of N fertilizer on plant agronomic indicators of inbred maize during 2014-2015 cropping.*

Agronomic indicator	Growth stage[1]	Fertilizer Treatment (2014-2015) 0	90	135	180	225	Means
		-------	-------	kg/ha	-------	-------	
Plant height (cm)	V8	135.0 ns[2]	144.5	136.3	144.0	137.1	139.4
	V10	163.4 a[3]	165.4 ab	172.6 c*	172.4 c	170.4 bc	--------[5]
	R1	161.1 ab	158.5 a	170.3 c*	169.3 c	167.0 bc	--------
	R2	162.5 ab	159.0 a	170.5 c*	170.5 c	168.6 bc	--------
SPAD-502	V6	37.4 a	38.8 ab	39.5 abc*	40.7 bc	41.9 c	--------
	V8	40.9 ns	43.41	44.7	43.8	43.6	43.3
	V10	42.4 a	46.1 b*[4]	47.2 b	46.4 b	47.9 b	--------
	R1	42.4 a	46.1 b*	47.2 b	46.4 b	47.9 b	--------
	R2	43.0 a	47.1 bc	45.6 b*	49.1 c	48.4 c	--------
NDVI (GreenSeeker)	V6	0.62 ns	0.56	0.63	0.64	0.60	0.61
	V8	0.71 ns	0.72	0.72	0.73	0.73	0.72
	V10	0.73 ns	0.73	0.72	0.74	0.74	0.73
	R1	0.73 ns	0.73	0.72	0.74	0.74	0.73
	R2	0.69 ns	0.70	0.68	0.70	0.68	0.69

[1]V6, V8, V10, R1 and R2 correspond to 31, 38, 45, 52 and 59 DAP, respectively.
[2]ns denotes that the treatments were not significantly different at p>0.05.
[3]Means for each agronomic indicator with different letters within a DAP are significantly different as determined with Fisher's LSD test (p<0.05).
[4]*No significant differences from this value onwards.
[5]Means were not calculated for significantly different data (p<0.05).

that NDVI values from V5 (25-35 DAP) and later stages were not significantly different.

Effects of cowpea cover crop

In the 2015-2016 season, seven out of 12 agronomic indicators had higher values with the cover crop rotation than the fallow treatment. The cowpea cover crop rotation had a stronger effect on agronomic indicators in the 2015-2016 season than in the 2014-2015 season (Table 6).

Espinosa-Irizarry (2016) observed that inbred maize under cowpea cover crop rotation was taller (as measured by plant height) and greener (as measured by SPAD-502). Rivera-Zayas et al. (2017) also reported a cover crop rotation effect on all three agronomic indicators ($p<0.05$). The higher plant height and SPAD-502 values for the 2015-2016 season with cover crop could be due to the greater amount of immediately available N (0-30 cm) found in the soils with cowpea rotation (Vilches-Ortega et al., 2022). Legumes such as cowpeas are known to increase soil N inputs through N_2 fixation (Havlin et al.,

TABLE 5.—*Effects of N fertilizer on plant agronomic indicators of inbred maize during 2015-2016 cropping.*

Agronomic indicator	Growth stage[1]	Fertilizer Treatment (2015-2016) 0	50	100	150	200	Means
		----------	----------	kg/ha	----------	----------	
Plant Height (cm)	V8	108.5 a[2]	119.3 b*	115.3 b	118.6 b	119.1 b	-------[4]
	V10	139.3 ns[3]	149.0	143.5	147.5	147.9	145.4
	R1	160.6 ns	170.6	165.4	166.3	167.3	166.0
	R2	175.6 ns	179.1	176.8	177.4	175.8	176.9
SPAD-502	V8	41.1 a	44.3 bc	42.7 ab	43.9 abc	45.8 c	--------
	V10	35.5 ns	38.6	37.1	38.8	41.1	38.2
	R1	36.7 ns	37.5	38.2	38.3	39.4	38.0
	R2	35.1 ns	36.7	34.0	36.2	41.9	36.8
NDVI (GreenSeeker)	V8	0.67 ns	0.67	0.68	0.69	0.69	0.68
	V10	0.71 ns	0.73	0.74	0.73	0.75	0.73
	R1	0.70 ns	0.7	0.71	0.71	0.71	0.71
	R2	0.61 ns	0.61	0.63	0.58	0.63	0.61

[1]V8, V10, R1 and R2 correspond to 35, 42, 49 and 56 DAP, respectively.
[2]Means for each agronomic indicator with different letters within a DAP are significantly different as determined with Fisher's LSD test ($p<0.05$).
[3]ns denotes that treatments were not significant at $p>0.05$.
[4]Means were not calculated for significantly different data ($p<0.05$).

2014; Kaspar and Singer, 2011; Karpenstein-Machan and Stuelpnagel, 2000; Uzoh et al., 2019).

Graphical plots and linear correlation analysis among the agronomic parameters measured and crop yield and crop N uptake were done to evaluate if these could be used as predictors of crop yield and crop N status. Previous research shows that these relationships are stronger when there is a wide range in crop yields because of varying N availability. Maximum maize yield of 7,234 kg/ha at N fertilizer rate of 135 kg/ha, and of 4,708 kg/ha at N fertilizer of 90 kg/ha was achieved for the 2014 and 2014-2015 seasons, respectively; N fertilizer did not affect maize yield in 2015-2016 season (Vilches et al., 2022). The fact that there were yield increases of only 12% and 27% with fertilization in 2014 and 2014-2015 may explain why most relationships were not significant, or there were only weak relationships. In 2014, significant linear relationships were obtained among plant height and yield for all growth stages, and for SPAD and crop N uptake at V10 and R1 stages. In 2014-2015 Greenseeker was significantly related to maize yield at V10 and R1 stages.

TABLE 6.—*Effects of cover crop rotation on plant agronomic indicators of inbred maize during 2014-2015 and 2015-2016.*

Agronomic Indicator	CC[4]	FA	Means
	------------------------2014-2015------------------------		
Plant Height 1[1]	141.5 ns[2]	137.3	139.4
Plant Height 2	169.1 ns	168.6	168.8
Plant Height 3	166.0 ns	164.5	165.2
Plant Height 4	167.1 ns	165.4	166.2
NDVI 1	0.61 ns	0.60	0.61
NDVI 2	0.73 ns	0.72	0.73
NDVI 3	0.74 b	0.72 a	--------[5]
NDVI 4	0.74 b	0.72 a	--------[5]
NDVI 5	0.70 ns	0.68	0.69
SPAD 1	39.8 ns	39.5	39.7
SPAD 2	43.8 b	42.7 a	--------[5]
SPAD 3	47.0 ns	45.0	46.0
SPAD 4	47.0 ns	45.0	46.0
SPAD 5	48.0 ns	45.3	46.6
	------------------------2015-2016------------------------		
Plant Height 1[1]	119.1 b[3]	113.3 a	--------[5]
Plant Height 2	149.0 b	141.9 a	--------[5]
Plant Height 3	170.1 b	162.0 a	--------[5]
Plant Height 4	179.4 ns	174.5	176.9
NDVI 1	0.69 ns	0.67	0.68
NDVI 2	0.74 b	0.72 a	--------[5]
NDVI 3	0.71 ns	0.70	0.71
NDVI 4	0.61 ns	0.61	0.61
NDVI 5	--------[6]	--------[6]	--------[5]
SPAD 1	45.5 b	41.6 a	--------[5]
SPAD 2	39.8 b	36.7 a	--------[5]
SPAD 3	40.1 b	36.0 a	--------[5]
SPAD 4	37.9 ns	35.7	36.8
SPAD 5	--------[6]	--------[6]	--------[5]

[1]Plant height (1 to 4) was recorded at 38, 45, 52 and 59 DAP in 2014-2015, respectively. NDVI (GreenSeeker) and SPAD-502 values (1 to 5) for 2014-2015 were taken at 31, 38, 45, 52 and 59 DAP, respectively. All three indicator measurements were determined at 35, 42, 49 and 56 DAP in 2015-2016, respectively.

[2]ns denotes that treatments were not significant at $p>0.05$.

[3]Means for each agronomic indicator with different letters within a DAP are significantly different as determined with Fisher's LSD test ($p<0.05$).

[4]Rotation factor, CC=cover crop and FA=fallow.

[5]Means were not calculated for significantly different data ($p<0.05$).

[6]NDVI (GreenSeeker) and SPAD data was not collected a fifth time for the 2015-2016 season.

CONCLUSIONS

The results of this experiment reveal that only some of the agronomic indicators were affected by N fertilizer rates and by the cover crop rotation. In the 2014 season, there was no significant fertilizer effect on SPAD-502 or on GreenSeeker-NDVI values and no fertilizer effect for GreenSeeker-NDVI values in the 2014-2015 and 2015-2016 seasons. Plant height was an important yield predictor in 2014. Overall SPAD-502 and plant height were sensitive to the effects of N fertilizer. Further work must be done to examine the lack of sensitivity of GreenSeeker-NDVI and the observed weak sensitivity of SPAD-502 to N fertilizer application. Plant height can be used as a yield predictor with higher confidence than SPAD-502. Although GreenSeeker-NDVI was not sensitive to N fertilizer, it managed to predict yield on two occasions. Further work should examine other limiting factors to maize growth which may have impeded obtaining more robust relationships among the agronomic parameters and inbred maize yield.

LITERATURE CITED

Espinosa-Irizarry, J., 2016. Efecto de la fertilización nitrogenada y cobertora sobre el rendimiento de una linea endogámica de maíz. University of Puerto Rico Mayagüez Campus, MSc Thesis. 65 pp. Available at: https://scholar.uprm.edu/handle/20.500.11801/40.

FAOSTAT, 2014. Food and Agriculture Organization of the United Nations. FAOSTAT Stat. database.

Garibaldi, L.A., B. Gemmill-Herren, R. D'Annolfo, B.E. Graeub, S.A. Cunningham and T.D. Breeze, 2017. Farming approaches for greater biodiversity, livelihoods, and food security. *Trends Ecol. Evol*. 32(1): 68-80.

Hargrove, W.L., D.E. Kissel and L.B. Fenn, 1961. Field measurements of ammonia volatilization from surface applications of ammonium salts to a calcareous soil. *Agron. J.* 69: 473-476.

Havlin, J.L., S.L. Tisdale, W.L. Nelson and J.D. Beaton, 2014. Agricultural productivity and environmental quality: pp 447-502, *In*: Soil Fertility and Fertilizers. 8th ed. Pearson Publishers. Upper Saddle River, NJ.

InfoStat, 2014. Grupo INFOSTAT, FCA, Universidad Nacional de Córdoba, Argentina.

Ju, T., G. Xing, X. Chen and S. Zhang, 2009. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proc. Natl. Acad. Sci*. 106(19): 3041-3046.

Kant, S., Y. Bi and S.J. Rothstein, 2011. Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *J. Exp. Bot.* 62(4): 1499-1509.

Karpenstein-Machan, M. and R. Stuelpnagel, 2000. Biomass yield and nitrogen fixation of legumes monocropped and intercropped with rye and rotation effects on a subsequent maize crop. *Plant and Soil* 218: 215-232.

Kaspar, T.C. and J.W Singer, 2011. The use of cover crops to manage soil: pp 321-337, *In*: J.L. Hatfield and T.J. Sauer (eds). Soil Management: Building a Stable Base for Agriculture. American Society of Agronomy and Soil Science Society of America, Madison, WI.

Meisinger, J.J., J.S. Schepers and W.R. Raun, 2008. Crop nitrogen requirement and fertilization: pp 563-612, *In*: J.S.Schepers and W.R. Raun (eds) Nitrogen in Agricultural Systems, Agronomy Monographs 49. ASA, CSSA, SSSA, Madison, WI.

Mengel, D., 2008. Enhancing nitrogen use efficiency in irrigated corn using sensor technology: pp 8-1 - 8-5, *In*: W.M. Stewart and W.B. Gordon (eds). Fertilizing for Irrigated Corn- Guide to Best Management Practices. International Plant Nutrition Institute (IPNI).

Muharam, F.M., K.F. Bronson, S.J. Maas and G.L. Ritchie, 2014. Inter-relationships of cotton plant height, canopy width, ground cover and plant nitrogen status indicators. *F. Crop. Res.* 169(3): 58-69.

Muñoz-Huerta, R.F., R.G. Guevara-González, L.M. Contreras-Medina, I. Torres-Pacheco, J. Prado-Olivarez and R.V. Ocampo-Velázquez, 2013. A review of methods for sensing the nitrogen status in plants. Advantages, disadvantages and recent advances. *Sensors* 13(8): 10823-10843. https://doi.org/10.3390/s130810823

Poehlman, J.M. and D.A. Sleper, 1995. Applications: Field crops utilizing hybrid breeding procedures: pp 321-344. *In*: Breeding Field Crops. Iowa State Press, Iowa.

Ranum, P., J.P. Peña-Rosas and M.N. Garcia-Casal, 2014. Global maize production, utilization, and consumption. *Ann. N. Y. Acad. Sci*. 1312(1): 105-112.

Raun, W.R. and G.V. Johnson, 1999. Improving nitrogen use efficiency for cereal production. *Agron. J*. 91: 357-363.

Rivera-Zayas, J., D. Sotomayor-Ramírez and R. Barnes, 2017. Nitrogen fertilizer rate for improving inbred maize (*Zea mays* L.) yield on the semi-arid southern coast of Puerto Rico. *J. Agric. Univ. P.R*. 101(2): 185-202. doi.org/10.46429/jaupr.v101i2.15789

Scharf, P.C., 2001. Soil and plant tests to predict optimum nitrogen rates for corn. *J. Plant Nutr*. 24: 805-826.

Shaver, T.M., R. Khosla and D.G. Westfall, 2011. Evaluation of two crop canopy sensors for nitrogen variability determination in irrigated maize. *Precis. Agric*. 12(6): 892-904.

Sotomayor-Ramírez, D., R. Huckaba, R. Barnes and J. Espinosa, 2012. Inbred maize response to cover crop and fertilizer-nitrogen. *J. Agric. Univ. P.R*. 96(1-2): 37-55. doi.org/10.46429/jaupr.v96i1-2.245

Tajul, M.I., M.M. Alam, S.M.M. Hossain, K. Naher, M.Y. Rafii and M.A. Latif, 2013. Influence of plant population and nitrogen-fertilizer at various levels on growth and growth efficiency of maize. *Sci. World J*. 1-9. DOI:10.1155/2013/193018

Tremblay, N., Z. Wang, B.-L. Ma, C. Belec and P. Vigneault, 2009. A comparison of crop data measured by two commercial sensors for variable-rate nitrogen application. *Precis. Agric*. 10(2): 145-161.

Uzoh, I.M., C.A. Igwe, C.B. Okebalama and O.O. Babalola, 2019. Legume-maize rotation effect on maize productivity and soil fertility parameters under selected agronomic practices in a sandy loam soil. *Scientific Reports* 9: 1-9.

Vilches-Ortega A., D. Sotomayor-Ramírez, J. Rivera-Zayas, and R. Barnes, 2022. Cover crop and nitrogen fertilizer influence on inbred maize performance and soil nitrogen. *J. Agric. Univ. P.R.* 106(1): 47-69.