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Predicting Nitrogen Fertilizer Needs for Rice in Arkansas Using Alkaline Hydrolyzable-Nitrogen

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The immediate profitability and long-term sustainability of domestic crop production have been threatened by increasing N fertilizer costs. Currently, there is no soil test which can accurately predict the N fertilizer needs of direct-seeded, delayed-flood rice (*Oryza sativa* L.) produced on silt loam soils in Arkansas. Fertilizer N recommendations could be improved substantially with a calibrated soil N test, while also lowering potential environmental impacts. Twenty-five N response trials were conducted between 2004 and 2008 to correlate alkaline-hydrolyzable N (AH-N), as quantified by the Illinois Soil N Test (ISNT) and direct steam distillation (DSD), with rice response parameters such as total N (TN) uptake, check plot grain yield, and percentage of relative grain yield (RGY) and calibrate AH-N to predict the fertilizer N rate required to achieve 95% RGY. Relationships with the selected parameters were evaluated for both methods over a series of soil depth increments that included 0 to 15, 15 to 30, 30 to 45, 45 to 60, 0 to 30, 0 to 45, and 0 to 60 cm using linear regression models. Alkaline hydrolyzable-N was significantly and positively correlated with all rice response parameters except check plot grain yield and percentage of RGY using AH-N at the 45- to 60-cm depth. Coefficients of determination were greatest for percentage of RGY at the 0- to 30- and 0- to 45-cm depth for the ISNT ($r^2 = 0.57$) and DSD ($r^2 = 0.73$), respectively. Calibration of the fertilizer N rate to achieve 95% RGY resulted in similar trends as the correlation of rice response parameters, but with higher r^2 values. Alkaline hydrolyzable-N explained 68 and 89% of the RGY variability in calibration for the ISNT using the 0- to 30-cm depth and the DSD using the 0- to 45-cm depth, respectively. These successful calibrations can be attributed to the N dynamics that exist in direct-seeded, delayed-flood rice production systems and identification of the proper sampling depth.

Abbreviations: AH-N, alkaline-hydrolyzable nitrogen; BIE, beginning internode elongation; DSD, direct steam distillation; EONR, economic optimum N rates; FNUE, fertilizer N-use efficiency; ISNT, Illinois Soil N Test; RGY, relative grain yield; TN, total nitrogen.

Soil testing is used routinely to guide fertilizer usage of P and K, and sometimes also for secondary and micronutrients, but there has been little success in the development of a soil test that can accurately predict potentially mineralizable soil-N. Requirements of a successful soil-based N test include; speed, efficiency, and the ability to correlate with crop N uptake and crop yield. Biological methods offer the highest predictive ability for estimation of potentially mineralizable-N, but are time-consuming (7–14 d) and inconsistent for predicting crop response (Stevenson and Cole, 1999). Chemical methods offer an attractive alternative due to relatively short analysis time, high reproducibility and correlation with biological estimations of potentially mineralizable-N. Unfortunately, interest in the chemical approach to soil N testing has long been limited by the prevailing view that no chemical method can accurately extract biologically labile organic-N (Wang et al., 2001).

Khan et al. (2001) rekindled the search for a chemical method that could accurately predict crop response to N with the development of the ISNT. This simple diffusion method was able to quantify AH-N, primarily in the form of amino sugars (Kwon et al., 2009; Roberts et al., 2009a), and predict when corn (*Zea mays* L.) would respond to N fertilizer application. Although the ISNT had shown promise

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for use in mainstream soil testing, criticism arose due to the high sample variability and inconsistencies with results (Klapwyk and Ketterings, 2005; Spargo et al., 2009). Several modifications were developed to address the initial problems with the ISNT, including sample rotation (15N Analysis Service, 2004), enclosed griddles (Klapwyk and Ketterings, 2005) and the use of an incubator (Spargo et al., 2009). Further work by Bushong et al. (2008) led to the development of a DSD technique as an alternative. The DSD technique has shown a strong correlation with the ISNT diffusion method while reducing analysis time and variability (Roberts et al., 2009a).

An essential attribute of any chemical soil-test method is the ability to correlate with crop response parameters such as TN uptake, check plot yield, and RGY with the definitive goal being the development of a calibration curve to predict N fertilizer needs based on soil test values. Originally the ISNT presented a "yes" or "no" answer as to when corn in Illinois would respond to the addition of N fertilizer (Khan et al., 2001). Substantial research efforts have focused on the utilization of the ISNT as a method to predict corn response and N fertilizer needs, but results have been mixed with limited success. Williams et al. (2007a, 2007b) successfully correlated and calibrated the ISNT for corn economic optimum N rates (EONR) in North Carolina and showed that the ISNT's predictive ability was enhanced when soils were categorized based on drainage classification. A study by Ruffo et al. (2006) identified ISNT as the single most important parameter evaluated in conjunction with site-specific yield-response functions. For an extensive set of on-farm response studies, Mulvaney et al. (2006) found that test values were positively related to check-plot yield and negatively related to delta yield, EONR and fertilizer N-use efficiency (FNUE) all at $p < 0.001$. Klapwyk and Ketterings (2006) were successful in using the ISNT to predict corn yield response to sidedress N in New York, but only when organic matter (OM) was considered. In contrast to these findings, work from several other states including Iowa (Barker et al., 2006b), Wisconsin (Osterhaus et al., 2008), Virginia (Spargo et al., 2009), Michigan, Minnesota, and Nebraska (Laboski et al., 2008) reported that the ISNT could not accurately predict corn response to N. The primary concerns raised in the latter studies involve the relationships between ISNT hydrolyzed-N and total N (TN), crop response to N and EONR. From these concerns emerged the view that the ISNT was not sensitive in measuring potentially mineralizable-N, but rather measured a constant fraction of TN, notwithstanding much evidence to the contrary from data reported by Barker et al. (2006a), Laboski et al. (2008), Osterhaus et al. (2008), and Spargo et al. (2009). Further evidence in the same direction has been provided by Roberts et al. (2009b) in that AH-N ranged from 11 to 38% of TN and there was no consistent relationship between the ratios of AH-N to TN with soil depth. No research has yet been conducted to correlate or calibrate DSD techniques with crop response, but the relationship to the ISNT and quantification of AH-N has been studied in detail (Sharifi et al., 2007; Bushong et al., 2008; Roberts et al., 2009a).

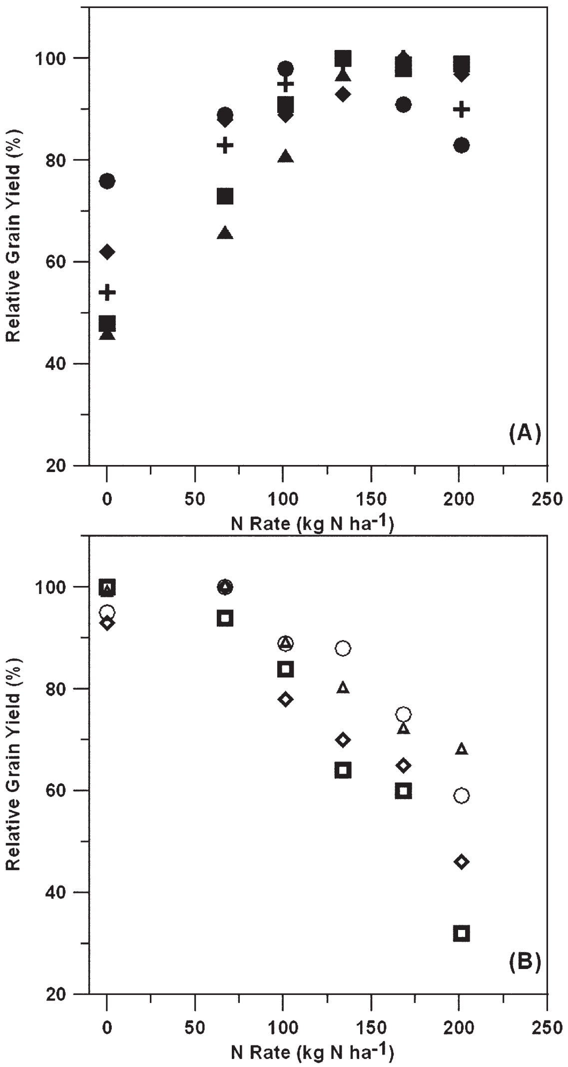

Fig. 1. Relationship between rice percentage of relative grain yield and N rate for sites that produce (A) positive response and (B) negative response due to the increasing addition of N fertilizer. Data adapted from Roberts et al. 2008.

Direct-seeded, delayed-flood rice represents an important commodity for many Midsouth states in the USA and is most often grown in rotation, usually with soybean [*Glycine max* (L.) Merr.] and occasionally with other crops or continuously (Norman et al., 2003). Arkansas is the primary rice producing state in the USA and harvests roughly 610,000 ha yr^{-1}. Current N fertilizer recommendations for rice in Arkansas are based on cultivar, previous crop, and soil texture (Wilson et al., 2001) and do not account for potentially mineralizable soil-N. Yield-goal based N fertilizer rate recommendations function on the basic assumption there is a distinct and positive relationship between the percentage of RGY and N fertilizer rate (Fig. 1A; data from Roberts et al., 2008). For many sites a positive relationship exists; unfortunately, sites with positive responses to N fertilizer do so to different degrees, depending on the native soil N available to the plant. Additionally, research has ascertained sites which mostly respond negatively to the addition of N fertilizer (Fig. 1B; data from Roberts et al., 2008). Every site responds differently to N fertilizer and without the development of new technology sites will continue to be fertilized improperly and result in negative economic and environmental impacts. Traditionally an acceptable soil-based N test has not existed that could consistently predict when sites would respond to the addition of fertilizer N, let alone to what degree. Nitrogen fertilizer rate recommendations made using the current system do not take into account the amount of N that is being supplied by the soil within a specific site or within a current season which can result in over or under application of N fertilizer. This in turn could cause economic losses due to reduced grain yields, increased disease susceptibility and lodging (Cartwright and Lee, 2001; Norman et al., 2006). Previous research has shown the importance of soil sampling depth on the correlation of the ISNT (Khan et al., 2001; Barker et al., 2006a) and how AH-N levels can significantly change with sampling depth (Mulvaney et al., 2006; Roberts et al., 2009b). Therefore, any research into the correlation and calibration of rice response with AH-N should be conducted over the entire rice rooting depth which has been reported as 60 cm by Beyrouty et al. (1987).

Identification of a soil test method that correlates with rice response parameters and can be calibrated for N fertilizer recommendations is becoming more important and will be essential for the immediate economic viability and long-term sustainability of rice production in Arkansas and other Midsouth states. Benefits of a soil-based N test are not limited to optimizing economic or agronomic returns, but include environmentally sound N fertilizer rate decisions. Therefore, the objectives of this study were to evaluate the ability of AH-N as quantified by the ISNT and DSD to: (i) correlate with rice N response parameters when no N fertilizer was applied and (ii) enable the calibration of N fertilizer rate to achieve near maximal yield (95% RGY) for rice grown across the array of eastern Arkansas silt loam soils.

MATERIALS AND METHODS

Nitrogen Fertilizer Rate Trials

Twenty-five N response trials were conducted on experiment stations and producer fields from 2004–2008 to determine the optimum N rate for direct-seeded, delayed-flood rice on silt loam soils in the primary rice producing regions of Arkansas. Selected soil data for the 25 sites are presented in Table 1, and include the soil series, soil classification, and previous crop. Soil fertility analysis (Mehlich-3 extractable nutrients, Helmke and Sparks, 1996) was performed on samples from each experimental site to ensure nutrients other than N were sufficient for optimum rice growth. Each site was fertilized with at least 40 and 90 kg K ha^{-1}, regardless of soil test recommendations to ensure these nutrients were not limiting and 11 kg Zn ha^{-1} were applied when either the soil test recommended a Zn application or there was a field history of Zn deficiency. Sites will be referred to as numbered in Table 1.

‘Wells’, ‘Francis’, ‘Jupiter’, and ‘Medark’ were the rice cultivars chosen for the N response trials and typically receive 168 kg N ha^{-1} when grown on silt loam soils in rotation with soybean (Wilson et al., 2001). Rice was drill-seeded at 120 kg ha^{-1} in plots that were nine rows wide (18 cm row spacing) by 4.9 or 6.1 m in length. In each of the N response studies, urea (46% N) was applied by hand in two-split applications; immediately before flooding (i.e., preflood) onto a dry soil surface and

Table 1. Study site and soil information for the rice N-response trials on silt loam soils.

Year	Site	Soil series	Soil classification	Previous crop	Location†
2004	1	Calhoun	fine-silty, mixed, active, thermic Typic Glossaqualf	soybean	ES
2004	2	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	ES
2004	3	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	ES
2004	4	Hilleman	fine-silty, mixed, active, thermic, albic Glossic Natraqualf	soybean	ES
2005	5	Calhoun	fine-silty, mixed, active, thermic Typic Glossaqualf	soybean	ES
2005	6	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	ES
2005	7	Hilleman	fine-silty, mixed, active, thermic, albic Glossic Natraqualf	soybean	ES
2006	8	Calloway	fine-silty, mixed, active, thermic Aquic Fraglossudualf	soybean	ES
2006	9	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	P
2006	10	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	ES
2006	11	Hilleman	fine-silty, mixed, active, thermic, albic Glossic Natraqualf	soybean	ES
2007	12	Calhoun	fine-silty, mixed, active, thermic Typic Glossaqualf	soybean	ES
2007	13	Foley	fine-silty, mixed, active, thermic, albic Glossic Natraqualf	catfish	P
2007	14	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	ES
2007	15	Hillemann	fine-silty, mixed, active, thermic, albic Glossic Natraqualf	soybean	P
2007	16	Dexter	fine-silty, mixed, active, thermic Ultic Hapludalf	fallow	P
2007	17	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	P
2007	18	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	P
2008	19	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	ES
2008	20	Calhoun	fine-silty, mixed, active, thermic Typic Glossaqualf	fallow	ES
2008	21	Calloway	fine-silty, mixed, active, thermic Aquic Fraglossudualf	rice	ES
2008	22	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	P
2008	23	Dewitt	fine smectitic, thermic Typic Albaqualf	soybean	P
2008	24	Hillemann	fine-silty, mixed, active, thermic, albic Glossic Natraqualf	soybean	ES
2008	25	Forestdale	fine, smectitic, thermic Typic Endoaqualf	fallow	ES

† Designates whether experiments were located on experiment stations (ES) or in production fields (P).

at beginning internode elongation (BIE), directly into the floodwater. The following total-N (preflood + BIE) rate splits were utilized at each location: 0, 68 (34 + 34), 100 (50 + 50), 134 (84 + 50), 168 (118 + 50) and 201 (151 + 50) kg N ha^{-1}, in a randomized complete block design with four replications. Following preflood N application, a 10-cm deep flood was established within 2 d and maintained until maturity. Urea applied preflood was treated with the urease inhibitor n-butyl-thiophosphoric triamide (NBPT) by blending 1 kg of prilled-urea with 4.2 mL of Agrotain solution (Agrotain International, St. Louis, MO). For sites established on experiment stations and producer fields, weeds and pests were controlled according to University of Arkansas Cooperative Extension Service recommendations using best management practices for direct-seeded, delayed-flood rice produced on silt loam soils.

Soil Sampling and Analysis

Soil samples were collected before the application of preflood N in the plots that were selected to receive no-N (control plots). A minimum of four soil cores were taken from each of the no-N plots to form a composite sample at depth increments of 0 to 15, 15 to 30, 30 to 45, and 45 to 60 cm. Samples were dried within 24 h of collection at 40°C and ground to pass through a 2-mm sieve (James and Wells, 1990). Soil series and classification were determined using Web Soil Survey; a product provided by the Natural Resources Conservation Service (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, 2008).

Soils were analyzed for NH_4–N, NO_3–N, and TN by the University of Arkansas Diagnostic Laboratory (Fayetteville, AR). Inorganic NH_4–N and NO_3–N were determined by colorimetric techniques according to Mulvaney (1996). Samples were analyzed for TN using an Elementar CN Variomax (Elementar Americas Inc., Mt. Laurel, NJ) according to the procedure of Nelson and Sommers (1996). Soil water pH was measured with a glass electrode in a 1:2 soil weight to water volume mixture. Alkaline hydrolyzable-N was determined using the ISNT-a modified microdiffusion technique utilizing 2 *M* NaOH and 5-h heating period (Khan et al., 2001) and a 10 *M* NaOH DSD method in which soil was distilled for ~7 min. (Bushong et al., 2008; Roberts et al., 2009a). Both the ISNT and 10 *M* DSD methods capture liberated NH_3 in a 40 g L^{-1} boric acid indicator solution and AH-N is quantified through titration to a predetermined endpoint. Analysis for AH-N was done in triplicate for each sample. Selected soil chemical characteristics for the 25 sites utilized in the study are presented in Table 2.

Rice Response Parameters

Rice tissue samples were collected at 50% heading from the no-N control plots by removing the aboveground portions of a 0.91-m linear

Table 2. Soil pH and selected N chemical characteristics by depth for the soils at the 25 sites.

Site	pH†				NH_4–N‡				NO_3–N‡				TN§			
					Soil sample depth (cm)											
	0–15	15–30	30–45	45–60	0–15	15–30	30–45	45–60	0–15	15–30	30–45	45–60	0–15	15–30	30–45	45–60
					mg N kg soil^{-1}											
1	7.5	7.3	6.9	6.9	19	6	6	7	3	3	1	< 1	1100	220	480	280
2	6.8	6.5	6.6	6.0	10	6	5	7	11	5	3	< 1	820	660	890	870
3	5.9	6.1	6.0	5.6	9	5	4	5	< 1	4	< 1	< 1	900	840	720	820
4	7.0	6.8	6.9	7.0	11	6	3	4	1	3	< 1	< 1	850	850	550	430
5	8.1	7.5	7.1	6.9	21	4	4	5	19	< 1	< 1	< 1	1030	470	390	470
6	6.7	6.5	6.2	6.3	20	4	4	7	6	3	< 1	1	1070	750	640	740
7	6.8	6.9	–	–	14	10	–	–	8	6	–	–	1000	960	–	–
8	7.0	7.2	6.9	6.1	9	7	5	5	10	8	8	7	870	450	330	350
9	6.0	6.5	6.3	5.8	5	2	2	3	9	4	4	2	880	760	790	730
10	5.7	6.1	6.1	5.8	6	4	3	4	10	8	5	4	680	600	490	590
11	7.2	6.9	7.3	7.6	4	2	2	2	4	4	2	2	890	570	370	450
12	6.7	6.5	5.6	5.6	3	1	2	2	20	10	8	6	883	462	554	489
13	6.9	7.5	7.5	7.6	40	168	132	97	76	21	16	7	1384	756	822	667
14	6.7	7.1	7.0	6.1	9	4	5	6	14	10	9	9	963	741	747	806
15	6.5	6.5	6.4	6.4	6	4	3	4	5	6	4	4	957	577	646	608
16	5.2	5.2	5.4	5.0	7	4	4	5	18	18	19	16	1366	604	646	539
17	6.7	7.1	7.1	5.9	8	6	6	10	17	13	13	9	799	715	639	734
18	6.6	6.6	5.3	5.3	8	4	5	6	16	13	11	12	1006	722	757	901
19	6.6	7.2	7.1	5.9	4	2	3	3	9	7	6	5	852	692	709	657
20	7.1	6.8	6.8	6.9	1	< 1	1	1	8	5	5	7	710	271	235	240
21	7.0	6.9	6.6	6.5	< 1	1	1	1	10	6	5	5	463	206	179	191
22	6.8	6.4	5.6	5.4	2	1	2	2	15	9	7	2	978	511	571	571
23	6.9	6.2	6.0	6.3	1	< 1	< 1	< 1	13	4	4	3	888	582	496	457
24	6.6	6.4	6.3	6.2	1	< 1	1	2	5	6	7	5	970	614	732	620
25	5.1	5.0	5.6	5.7	4	1	2	1	19	12	9	6	1542	765	489	439

† Soil/water ratio, 1:2.

‡ Determined by salicylate colorimetric techniques, Mulvaney (1996).

§ Total nitrogen (TN) determined by dry combustion technique, Nelson and Sommers (1996).

section of an inner row from each plot (Norman et al., 1992). Tissue samples were oven dried at 60°C to a constant weight, weighed and ground to pass through a 1-mm sieve. A subsample was sent to the University of Arkansas Diagnostic Laboratory (Fayetteville, AR) and TN for plant tissue was determined by combustion (Campbell, 1992). Total N uptake (in kg N ha^{-1}) was determined as the product of the plant TN concentration of the rice tissue and the dry weight and extrapolated to an area basis. At maturity, the four center rows of each plot were harvested, the moisture content and weight of the grain determined, and yields expressed as kg rough rice ha^{-1} on a 120 g kg^{-1} moisture basis. Relative grain yield was determined by dividing the rice yield of the no-N control plot by the maximum yield achieved at that site (the highest mean yield for a specific N rate within a site), multiplied by 100. The N rate to achieve 95% RGY was determined using a regression model fit to yield response for each of the individual sites studied. The rice response parameters for each site are listed in Table 3.

Statistical Analysis

All statistical analyses were performed using JMP 7.0 (SAS Institute, Inc., Cary, NC). To obtain values for the 0- to 30-, 0- to 45-, and 0- to 60-cm depths for each site, values for individual depths were summed and divided by the number of depths used in the summation. For example, the value for the 0- to 30-cm depth represents the mean value of the 0- to 15- and 15- to 30-cm depths for that site (a constant bulk density was assumed in summing soil test values for different depths). For the ISNT and DSD procedures, mean values from triplicate analyses for each depth (0–15, 15–30, 30–45, 45–60, 0–30, 0–45, and 0–60 cm) were regressed against the mean rice response of interest. Means for TN uptake from the no-N control plots, mean rice grain yield, and mean RGY were calculated across replicates for each site-year. Nitrogen rates required to achieve 95% relative grain yield were determined using the profiler function in JMP 7.0, which incorporates the quadratic equation from the N response curve generated for each site-year to calculate the corresponding N rate. Calibration of each method was achieved by regressing the N rate to achieve 95% RGY for each site-year versus the AH-N value of the soil as determined by the ISNT and DSD for each of the depths. Linear and quadratic models were considered for each of the correlations of the AH-N and for the calibration of 95% RGY vs. AH-N, but the quadratic term was not significant in any model and therefore only linear relationships were considered and included for discussion. The number of site years included for correlation of TN uptake from the no-N control plots, mean rice grain yield, and mean RGY are 25 for the 0- to 15-, 15- to 30-, and 0- to 30-cm depths and 24 for all other correlations due to the lack of samples for Site 7 at depths <30 cm. A

Table 3. Mean and standard deviations for alkaline hydrolyzable-N (AH-N) as determined by the Illinois Soil Nitrogen Test (ISNT) and direct steam distillation (DSD) for each soil depth increment; total N uptake, check plot grain yield, and percentage of relative grain yield (RGY) of rice receiving no N; and the N rate predicted to achieve 95% RGY for the 25 sites utilized in the study.

Site	ISNT†				DSD†				Total N uptake‡	Check plot yield‡	RGY‡	N rate to achieve 95% RGY‡
	Soil sample depth (cm)											
	0–15	15–30	30–45	45–60	0–15	15–30	30–45	45–60				
	-mg N kg soil^{-1}-								kg N ha^{-1}	kg rice ha^{-1}	%	kg N ha^{-1}
1	141 ± 7.0	45 ± 5.4	42 ± 4.3	36 ± 2.1	156 ± 6.7	66 ± 4.1	60 ± 4.0	42 ± 1.5	53	4710	44	150
2	134 ± 3.4	84 ± 2.8	124 ± 2.7	134 ± 3.5	136 ± 4.0	94 ± 1.8	136 ± 3.6	146 ± 2.1	147	8948	83	58
3	118 ± 7.7	96 ± 6.8	101 ± 7.1	109 ± 5.3	127 ± 2.1	109 ± 2.4	100 ± 1.4	116 ± 1.6	100	6882	61	114
4	144 ± 8.8	96 ± 8.6	42 ± 9.1	38 ± 4.2	151 ± 5.4	103 ± 4.8	55 ± 1.3	47 ± 0.9	46	3921	48	84
5	136 ± 2.1	50 ± 1.7	39 ± 1.1	35 ± 0.9	144 ± 1.9	78 ± 0.7	51 ± 0.8	51 ± 1.1	45	4389	40	138
6	148 ± 3.0	78 ± 2.9	77 ± 2.1	113 ± 1.6	157 ± 2.2	81 ± 2.6	92 ± 5.3	118 ± 1.2	63	4106	37	100
7	151 ± 8.0	109 ± 4.2	–	–	155 ± 5.7	137 ± 2.9	–	–	81	6392	74	73
8	99 ± 6.8	59 ± 5.1	43 ± 2.3	43 ± 2.8	113 ± 5.7	77 ± 2.1	56 ± 1.6	54 ± 1.8	54	2810	29	177
9	107 ± 6.3	93 ± 4.9	97 ± 3.8	91 ± 5.1	115 ± 3.0	101 ± 4.2	105 ± 2.6	103 ± 3.5	119	3717	62	104
10	104 ± 7.9	84 ± 4.2	76 ± 3.5	92 ± 6.2	111 ± 6.0	95 ± 4.9	88 ± 5.2	98 ± 3.9	81	2949	38	109
11	127 ± 7.4	77 ± 3.9	48 ± 1.6	44 ± 2.0	132 ± 5.9	90 ± 4.5	63 ± 2.4	59 ± 1.4	75	4393	49	141
12	131 ± 5.6	73 ± 4.9	60 ± 4.6	50 ± 3.2	131 ± 4.0	83 ± 2.1	70 ± 1.6	59 ± 1.0	62	4385	43	155
13	181 ± 8.2	240 ± 11	211 ± 9.8	165 ± 6.3	210 ± 5.3	280 ± 7.1	243 ± 6.9	205 ± 4.3	153	9321	100	0
14	137 ± 5.9	97 ± 4.1	100 ± 4.4	111 ± 4.8	143 ± 5.8	107 ± 5.5	102 ± 4.9	115 ± 3.7	102	4413	46	100
15	161 ± 6.1	112 ± 5.8	104 ± 3.4	112 ± 5.1	152 ± 3.7	110 ± 2.6	109 ± 2.3	108 ± 2.1	121	7006	74	92
16	217 ± 9.2	91 ± 4.1	95 ± 3.9	84 ± 2.8	230 ± 7.6	106 ± 3.3	108 ± 4.5	85 ± 3.5	156	9878	93	19
17	137 ± 3.6	99 ± 2.7	120 ± 2.3	134 ± 2.0	146 ± 4.5	105 ± 3.9	103 ± 3.4	129 ± 3.1	98	6854	61	95
18	138 ± 8.4	96 ± 6.5	95 ± 5.9	120 ± 5.6	141 ± 4.6	98 ± 4.9	124 ± 5.6	141 ± 3.4	119	7052	71	77
19	113 ± 7.6	93 ± 6.8	105 ± 4.2	96 ± 5.7	130 ± 8.1	111 ± 7.3	117 ± 6.8	114 ± 5.9	71	3830	48	104
20	97 ± 3.2	44 ± 4.6	37 ± 1.0	28 ± 0.8	122 ± 4.2	72 ± 3.5	63 ± 2.0	57 ± 1.1	64	3982	37	146
21	61 ± 3.2	28 ± 0.8	26 ± 0.6	22 ± 0.5	91 ± 2.1	59 ± 0.7	54 ± 1.1	51 ± 1.2	31	1966	19	185
22	102 ± 6.7	59 ± 3.1	62 ± 4.5	49 ± 3.2	133 ± 4.6	102 ± 5.2	88 ± 4.1	82 ± 5.6	90	6905	61	119
23	119 ± 7.2	59 ± 5.4	71 ± 5.0	93 ± 4.6	149 ± 3.1	97 ± 3.6	106 ± 4.1	121 ± 3.0	81	5494	49	101
24	123 ± 5.2	80 ± 3.4	97 ± 3.8	86 ± 6.4	143 ± 5.8	110 ± 6.4	116 ± 2.4	105 ± 3.7	53	4133	52	95
25	177 ± 9.1	78 ± 6.3	42 ± 5.7	35 ± 6.7	212 ± 7.2	111 ± 4.3	77 ± 4.9	67 ± 4.0	170	9223	96	41

† Mean of three replicate determinations.

‡ Mean of four replicate determinations.

similar trend is seen for the calibration of 95% RGY vs. AH-N, but the number of sites was reduced by one because the non-responsive site (13) was excluded from the calibration data.

RESULTS

Alkaline hydrolyzable-N ranged from 22 to 240 mg N kg soil^{-1} for ISNT and from 42 to 280 mg N kg soil^{-1} for the DSD over all sites and soil depths (Table 3) and is significantly lower than AH-N levels reported by researchers in the upper Midwest (Barker et al., 2006a; Mulvaney et al., 2006; Laboski et al., 2008) but similar to values reported in the Mid-Atlantic USA (Williams et al., 2007a; Spargo et al., 2009). Sites were selected to reflect the range of silt loam soil series on which rice is grown in Arkansas, as well as typical management histories. A significant difference among AH-N concentrations was measured based on soil series, history in rice, previous crop and the depth of soil being analyzed. Subsoil N mineralization was addressed by Mulvaney et al. (2006) and suggested that depths > 30 cm could influence crop response to fertilizer N. These results are similar to the findings of Roberts et al. (2009b), which indicated the significant differences in AH-N concentrations with soil depth and the potential for crop response to N fertilizer to be influenced by potentially mineralizable-N at soil depths > 30 cm. There was no consistent trend in AH-N levels with soil depth and the highest concentrations were not always found in the top 15 cm of the soil profile. Alkaline hydrolyzable-N quantified by DSD was consistently higher than that quantified by the ISNT and reflects the results of previous work by Bushong et al. (2008), but this data set reflects that same trend over each of the four soil depth increments.

Results of the N rate trials indicated that of the 25 harvested site-years, 24 had a significant and positive response to fertilizer N when compared with the no N control and a single site-year (13) where no response to N fertilizer was identified. The range of AH-N and TN values across the primary silt loam rice-producing areas of Arkansas resulted in a wide range of N response, but little success in the identification of non-responsive sites. Check plot grain yields ranged from 1966 to 9878 kg rice ha^{-1}, while RGY values ranged from 19 to 100% of the maximal yield for a given site. Predicted fertilizer N rates required to maximize yield at each site varied greatly from 0 to 185 kg N ha^{-1}, and several sites (13, 16, 24, and 25) indicated significant yield reductions when excess N fertilizer was applied.

Correlation with Rice Nitrogen Response

The AH-N values for each of the soil tests (ISNT or DSD) and each of the soil depth increments (0–15, 15–30, 30–45, and 45–60 cm) as well as the average AH-N values for cumulative depths (0–30, 0–45 and 0–60 cm) were evaluated for relationships with rice response. The wide variation in AH-N values with soil depth resulted in varying levels of correlation with rice response based on whether a specific depth increment or a cumulative approach was utilized. Based on the similarity of the soil texture and that drainage classification is not as important for delayed-flood rice production, all site-years were compared and not broken into subgroups, as seen in previous work with the ISNT in North Carolina (Williams et al., 2007a).

The relationship between AH-N values and rice TN uptake, check plot grain yield and percentage of RGY are shown in Table 4. A significant positive correlation between AH-N, quantified by the ISNT and DSD, and TN uptake was seen for all soil depth increments. The highest r^2 value for the ISNT and TN uptake correlations was 0.48 at the 45- to 60- and 0- to 30-cm depth increments. Similar values of 0.41, 0.46, and 0.47 relating to the 0- to 15-, 0- to 60-, and 0- to 45-cm depths, respectively were also found for the correlation between TN uptake and ISNT. A similar trend was seen when TN uptake was correlated with DSD, with the highest r^2 values, 0.48 and 0.46, occurring with the 0- to 60- and 0- to 45-cm depths, respectively. All regression models for AH-N correlations with TN uptake were highly significant, but resulted in relatively low coefficients of determination (<0.50).

Correlation of check plot yield with AH-N resulted in significant linear regression models for all depths analyzed except the 45- to 60-cm depth for both the ISNT and DSD (Table 4). The best predictive relationship for ISNT, was found at the 0- to 15-cm depth where the r^2 value was 0.51, but the highest r^2 for DSD was found for the 0- to 45-cm depth. Although there were similar trends in the ISNT and DSD check plot yield was significantly and positively correlated with AH-N, but the DSD resulted in consistently higher r^2 values compared with the ISNT. For the silt loam soils analyzed in this study, the reduced sample to sample variability of the DSD method enhanced its predictive ability and is supported by the findings of Roberts et al. (2009a). Although check plot yield has been used as a response parameter to evaluate the effectiveness of soil test correlation, many factors (planting date, environment, soil test P, K, and Zn concentrations, fertilizer rate, etc.) can influence actual rice yield other than the components in question (e.g., AH-N concentrations and N rate). Therefore, the percentage of RGY was also considered and represents a more logical approach due to the fact that the data have been normalized before correlation with AH-N.

Regression models relating the percentage of RGY to AH-N were highly significant and positively correlated for all soil depths except the 45- to 60-cm depth as was seen for check plot yield (Table 4). Coefficients of determination were higher than those obtained for either TN uptake or check plot yield and ranged from 0.25 to 0.57 and 0.33 to 0.73 for the ISNT and DSD, respectively. The highest r^2 value (0.57) for the ISNT and the percentage of RGY was found for the 0- to 30-cm depth (Fig. 2). The r^2 values for the correlation between DSD and percentage of RGY were similar to those found for the ISNT, but were considerably higher for the 0- to 45-cm depth which resulted in the highest r^2 for all rice response correlations at 0.73 (Fig. 3).

Alkaline hydrolyzable-N was positively and significantly correlated with TN uptake, check plot yield and the percentage of RGY for all soil depth increments except the 45- to 60-cm

Table 4. Regression models for the relationship between total N uptake, check plot grain yield and percentage of relative grain yield (RGY) with alkaline hydrolyzable-N (AH-N) quantified by the Illinois Soil Nitrogen Test (ISNT) and direct steam distillation (DSD).

Relationship	Soil sample depth	ISNT			DSD		
		Regression model	n¶	r^2	Regression model	n¶	r^2
	cm						
Total N uptake†	0–15	$-12.9 + 0.77x$	25	0.41***	$4.87 + 0.60x$	25	0.40 ***
	15–30	$42.8 + 0.55x$	25	0.31**	$39.3 + 0.48x$	25	0.26**
	30–45	$43.6 + 0.58x$	24	0.36**	$31.9 + 0.61x$	24	0.39**
	45–60	$47.2 + 0.53x$	24	0.48***	$36.1 + 0.57x$	24	0.33**
	0–30	$-3.3 + 0.86x$	24	0.48***	$-0.2 + 0.74x$	24	0.45***
	0–45	$5.0 + 0.86x$	24	0.47***	$-2.5 + 0.81x$	24	0.46***
	0–60	$13.6 + 0.81x$	24	0.46***	$4.1 + 0.79x$	24	0.48***
Check Plot Yield‡	0–15	$-1058 + 49.2x$	24	0.51***	$84 + 37.8x$	24	0.49***
	15–30	$2299 + 38.9x$	24	0.17*	$-378 + 59.7x$	24	0.25*
	30–45	$3011 + 30.9x$	23	0.18*	$1531 + 42.4x$	23	0.26*
	45–60	$37 + 21.3x$	23	0.14	$3029 + 25.3x$	23	0.15
	0–30	$1222 + 63.2x$	23	0.45***	$-1930 + 62.1x$	23	0.52***
	0–45	$955 + 66.5x$	23	0.42***	$-4757 + 92.8x$	23	0.61***
	0–60	$765 + 50.9x$	23	0.34**	$-1663 + 67.7x$	23	0.48***
% RGY§	0–15	$-5.5 + 0.46x$	24	0.53***	$5.5 + 0.35x$	24	0.50***
	15–30	$14.5 + 0.51x$	24	0.34**	$-16.5 + 0.74x$	24	0.45***
	30–45	$30.2 + 0.32x$	23	0.25*	$15.0 + 0.44x$	23	0.33**
	45–60	$38.5 + 0.20x$	23	0.15	$32.5 + 0.24x$	23	0.16
	0–30	$-12.7 + 0.65x$	23	0.57***	$-17.0 + 0.61x$	23	0.60***
	0–45	$-10.2 + 0.68x$	23	0.53***	$-46.4 + 0.93x$	23	0.73***
	0–60	$8.2 + 0.51x$	23	0.42***	$-14.7 + 0.67x$	23	0.57***

* Statistical significance at $p < 0.05$ levels.

** Statistical significance at $p < 0.01$ levels.

*** Statistical significance at $p < 0.001$ levels.

† x, Alkaline hydrolyzable-N (mg N kg soil^{-1}); y, total N uptake from check plots (kg N ha^{-1}).

‡ x, Alkaline hydrolyzable-N (mg N kg soil^{-1}); y, check plot grain yield (kg rice ha^{-1}).

§ x, Alkaline hydrolyzable-N (mg N kg soil^{-1}); y, percentage of relative grain yield (%RGY).

¶ n, number of sites.

depth for check plot yield and the percentage of RGY (Table 4). Although both methods were able to capture the relationship between AH-N concentration and rice response, the DSD r^2 values were consistently higher. The predictive ability of DSD may be enhanced by the fact that the sample to sample variability of the DSD is considerably lower than that of the ISNT as seen by the standard deviations reported in Table 3 and the work by Bushong et al. (2008). An increase with cumulative sampling depth in r^2 values for both the ISNT and DSD suggests that potentially mineralizable-N found deeper than 30 cm may significantly contribute to N uptake and rice growth (Mulvaney et al., 2006). The inability of AH-N values to correlate with check plot yield and percentage of RGY at the 45- to 60-cm depth was reflected in lower r^2 values for the 0- to 60-cm depth and suggest that the high correlations at the 0- to 30- and the 0- to 45-cm depths are real and not an artifact of "dilution".

Calibration of Nitrogen Rate Using Alkaline Hydrolyzable-Nitrogen

Based on the results from the response trials a wide range of N rates was required to achieve 95% RGY, with only Site 13 not responding to the addition of N fertilizer (Table 3) (Sims, 1964). Site 13 followed cat-

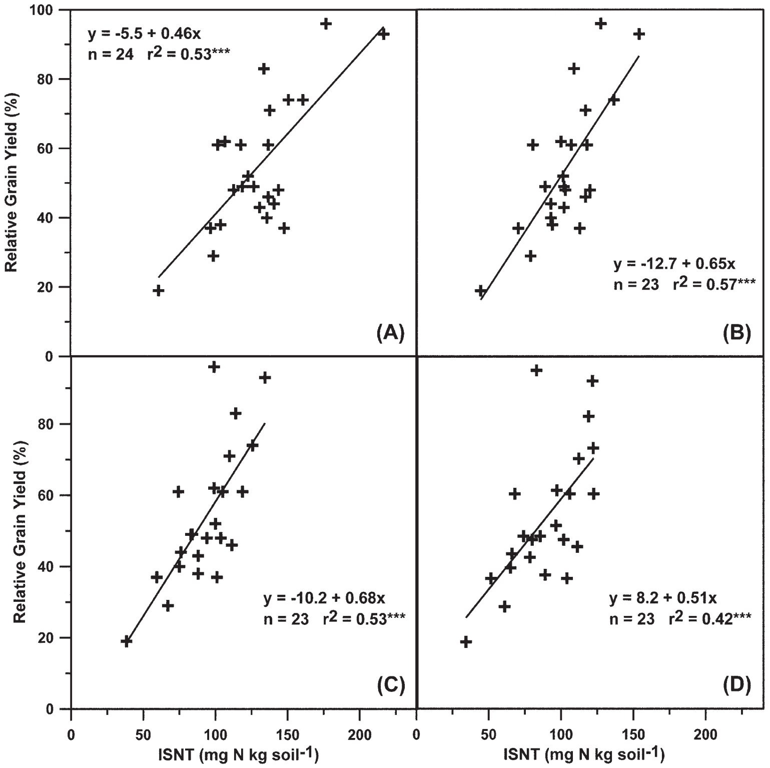

Fig. 2. Correlation of percentage of relative grain yield (RGY) of the rice receiving no N versus alkaline hydrolyzable-N (AH-N) as determined by the Illinois Soil Nitrogen Test (ISNT) for the four cumulative depths (A) 0 to 15 cm, (B) 0 to 30 cm (C) 0 to 45 cm, and (D) 0 to 60 cm, respectively. All correlations were significant at the 0.001 level of probability.

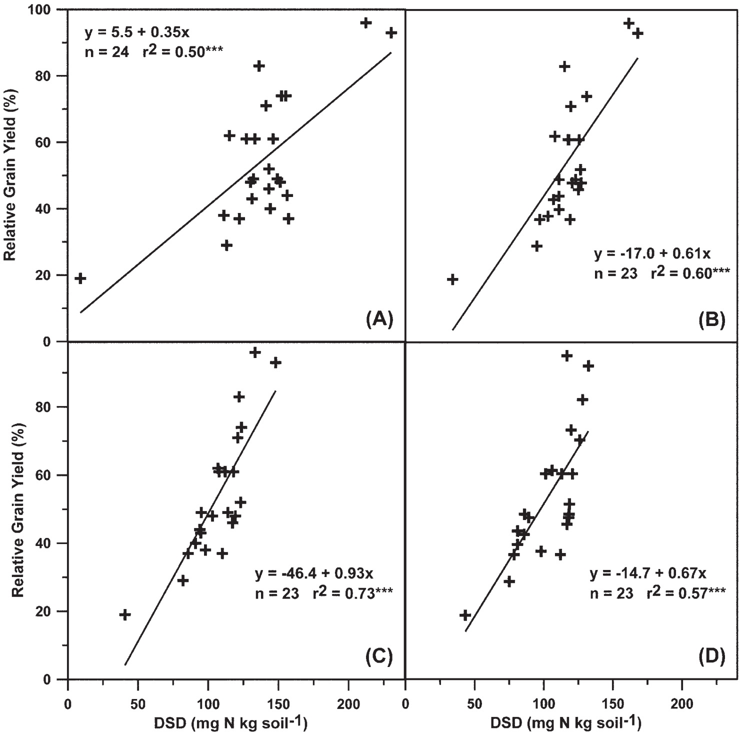

Fig. 3. Correlation of percentage of relative grain yield (RGY) of the rice receiving no N versus alkaline hydrolyzable-N (AH-N) as determined by Direct Steam Distillation (DSD) for the four cumulative depths (A) 0 to 15 cm, (B) 0 to 30 cm, (C) 0 to 45 cm, and (D) 0 to 60 cm, respectively. All correlations were significant at the 0.001 level of probability.

fish in the crop rotation (Table 1), which resulted in high levels of AH-N (Table 3) as well as exchangeable NH_4–N (Table 2). Non-responsive sites were relatively hard to find across the silt loam rice producing areas of Arkansas and limited the dataset for sites not requiring N fertilizer. To calibrate the N fertilizer needs to achieve 95% RGY with AH-N only the 24 responsive sites were included and predicted optimum N rates ranged from 19 to 185 kg N ha^{-1} (Table 3). The site that required the highest N rate followed rice in the rotation (Tables 1 and 3) and it is well established that rice following rice requires a higher N rate to optimize yields than rice following soybean due to the high level of N immobilization during decomposition of highly carbonaceous rice residues (Norman et al., 2003). The sites requiring the least amount of N were previously amended with biosolids and fallowed which allowed native N fertility to increase before rice cultivation. Although the present dataset includes only a single site that was non-responsive to N fertilization, four sites required <60 kg N ha^{-1} to achieve 95% RGY and there was a wide enough range of N response to facilitate the development of a fairly robust calibration curve.

Calibration of N fertilizer needs for rice produced on silt loam soils was achieved by regressing the N rate required to achieve 95% RGY versus the AH-N concentration quantified by either the ISNT or DSD. The N rate required to achieve 95% RGY was significantly and negatively correlated with AH-N concentration (Table 5) for both analytical methods (ISNT and DSD) and all depth increments analyzed (0–15, 15–30, 30–45, 45–60, 0–30, 0–45, and 0–60 cm). The trends identified in correlation of rice response to increasing AH-N levels are reflected here and mimic the relationships seen for the analytical methods as well as the depth increments. Coefficients of determination increased with increasing cumulative depth and the highest r^2 for the ISNT was 0.68 for the 0- to 30-cm depth, but the 0- to 15-, 0- to 45-, and 0- to 60-cm depths all had highly significant correlations with r^2 values of 0.58, 0.66, and 0.57, respectively (Fig. 4 and Table 5). The highest r^2 values for calibration of N rate for the ISNT analytical method occurred at the same soil depth as the highest values for all of the rice response parameters including TN uptake, check plot grain yield and the percentage of RGY. Although the significant calibration of N rate with AH-N is promising, the r^2 values were not as high as values reported in the Mid-Atlantic USA for the calibration of corn and EONR, but these high values were achieved by separating soils into drainage classifications (Williams et al., 2007a). Soil drainage class may not influence the response of direct-seeded, delayed-flood rice. Researchers in New York have utilized organic matter as a correction factor to improve the predictive ability of the ISNT (Klapwyk and Ketterings, 2006), but this approach was not evaluated here.

Table 5. Regression models for the relationship between the N rate to achieve 95% relative grain yield (95% RGY) with alkaline hydrolyzable-N (AH-N) quantified by the Illinois Soil Nitrogen Test (ISNT) and direct steam distillation (DSD).

Relationship	Soil sample depth	ISNT			DSD		
		Regression model†	n¶	r^2	Regression model†	n¶	r^2
	cm						
95% RGY	0–15	237– 1.00*x*	24	0.58***	214– 0.77*x*	24	0.56 ***
	15–30	202– 1.21*x*	24	0.45***	264– 1.64*x*	24	0.52**
	30–45	165– 0.76*x*	23	0.32**	200– 1.03*x*	23	0.43**
	45–60	152– 0.57*x*	23	0.28*	167– 0.65*x*	23	0.28**
	0–30	259– 1.45*x*	23	0.68***	265– 1.34*x*	23	0.69***
	0–45	256– 1.57*x*	23	0.66***	337– 2.10*x*	23	0.89***
	0–60	219– 1.23*x*	23	0.57***	269– 1.56*x*	23	0.73***

*Statistical significance at $p < 0.05$ level.

**Statistical significance at $p < 0.01$ level.

*** Statistical significance at $p < 0.001$ level.

† x, Alkaline hydrolyzable-N (mg N kg $soil^{-1}$); y, N fertilizer rate to achieve 95% relative grain yield (95%RGY) (kg N ha^{-1}).

¶ n, number of sites.

To our knowledge there has been no other investigation of the ability of DSD to predict optimum N fertilizer rates. Much like the results reported for correlation of rice response parameters with DSD, the calibration of N rate with AH-N quantified by DSD resulted in highly significant and negative correlations (Table 5). Coefficients of determination were numerically higher for

DSD than ISNT and were similar in trend to the correlation of DSD with rice response parameters (Table 4). Coefficients of determination increased with increasing cumulative depth to 45 cm, but then dropped slightly at the 0- to 60-cm depth increment (Table 5; Fig. 5). The highest r^2 value for the calibration of N rate occurred with the 0- to 45-cm depth increment of AH-N and explained 89% of the variability in the RGY. The DSD's ability to predict the N rate required to achieve 95% RGY was highest with the 0 to 45 cm of AH-N and coincided with the highest r^2 values for both check plot yield and percentage of RGY. When compared with the ISNT, the r^2 values for N rate calibration were similar for all depths except the 0- to 45- and 0- to 60-cm depths, but were not compared statistically. The predictive ability of the DSD calibration curve was about 20% higher than the ISNT for the 0- to 45- and 0- to 60-cm depths. As previously indicated the sample to sample variability of the DSD is considerably lower than the ISNT due to the nature of the methodology and may have contributed to the high r^2 values seen for N rate calibration to achieve 95% RGY. Differences in the types of organic N compounds being quantified by the ISNT and DSD (Roberts et al., 2009a) methods may also contribute to the differences observed in the ability of these two methods to predict N fertilizer needs for direct-seeded, delayed-flood rice.

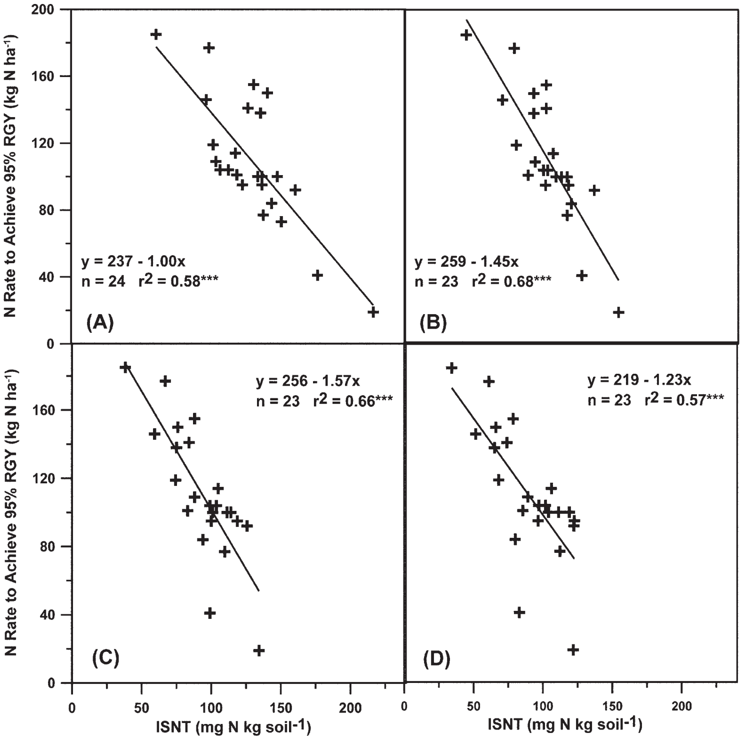

Fig. 4. Calibration curve for the N rate to achieve 95% relative grain yield (RGY) utilizing alkaline hydrolyzable-N (AH-N) as determined by the Illinois Soil Nitrogen Test (ISNT) for the four cumulative depths (A) 0 to 15 cm, (B) 0 to 30 cm (C) 0 to 45 cm, and (D) 0 to 60 cm, respectively. All correlations were significant at the 0.001 level of probability.

DISCUSSION

The relationships between AH-N quantified by the ISNT and DSD are specific for rice grown on silt loam soils in Arkansas and suggest that these two methods have the ability to predict rice responses such as TN uptake, check plot grain yield and the percentage of RGY. Calibration curves were developed that identified the ability of AH-N to predict the N rate required to achieve 95% RGY with the highest coefficients of determination occurring at the 0- to 30-cm depth for the ISNT (r^2 = 0.68) and the 0- to 45-cm depth for the DSD method (r^2 = 0.89). These results are similar to the findings for ISNT presented by other researchers for the calibration of N rate for corn (Klapwyk and Ketterings, 2006; Williams et al., 2007a). Previous work with the ISNT has resulted in a split decision on the method's predictive ability for N rate recommendations with several studies concluding that the ISNT was not an acceptable indicator for potentially mineralizable-N (Barker et al., 2006a; Laboski et al., 2008; Osterhaus et al., 2008; Spargo et al., 2009). Many of the negative findings associated with the ISNT have come from studies where no attempt was made to account for confounding factors that influence soil N availability or crop N requirement. As discussed by Mulvaney et al. (2006), such factors include soil moisture and temperature, differing climatic and soil conditions over broad geographical ar-

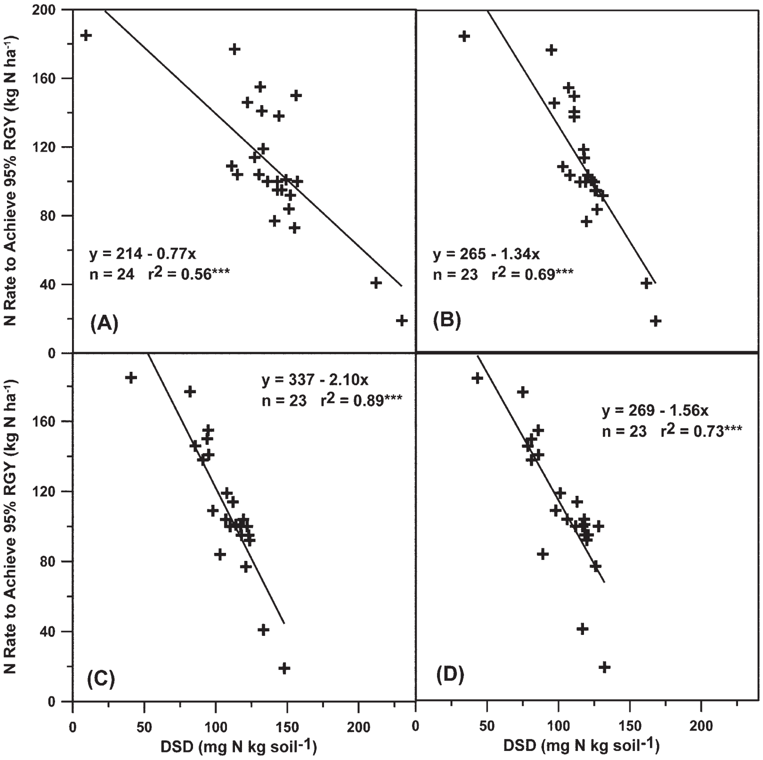

Fig. 5. Calibration curve for the N rate to achieve 95% relative grain yield (RGY) utilizing alkaline hydrolyzable-N (AH-N) as determined by direct steam distillation (DSD) for the four cumulative depths (A) 0 to 15 cm, (B) 0 to 30 cm, (C) 0 to 45 cm, and (D) 0 to 60 cm, respectively. All correlations were significant at the 0.001 level of probability.

eas, plant population, residue management, subsoil fertility and soil acidity.

Explanations as to why AH-N quantified by DSD in this study achieved such a high coefficient of determination and described the variability in the N rate data for rice include, but are not limited to: (i) direct-seeded, delayed-flood rice production boasts one of the highest and predictable N fertilizer use efficiencies in the world, (ii) N mineralization rate in direct-seeded, delayed-flood rice production is relatively constant under flooded conditions, (iii) correlation and calibration that considered the entire rooting depth of the crop, and (iv) greater precision and perhaps more accuracy than the ISNT in measured AH-N.

The consistency of the production system is a key concern when attempting to correlate and calibrate a N soil test that estimates the amount of N supplied by the soil. Direct-seeded, delayed-flood rice production has reported high rates of N fertilizer efficiency, 65 to 75% for the aboveground biomass (Wilson et al., 1989; Norman et al., 1992; Guindo et al., 1994; Wilson et al., 1994; Bufogle et al., 1997), but more important than the rate of efficiency is the consistency. High variability in the N fertilizer use efficiency across sites can lower the ability of a soil test to correlate with crop response, but the predictive ability of a soil test is greatly improved when the N fertilizer efficiency is consistent across sites.

Properly managed direct-seeded, delayed-flood rice production systems significantly limit the potential for N fertilizer losses, due to the use of ammonium based fertilizers and timely flooding to prevent nitrification/denitrification (Norman et al., 2003). Mineralization of soil N can influence the ability of a soil N test to correlate with crop response and is influenced by temperature, moisture, and aeration. In direct-seed, delayed-flood rice the establishment and management of a permanent flood helps to moderate temperature and maintain relatively consistent moisture and oxygen levels, thereby generating a consistent N mineralization rate.

Proper soil sampling depth and analytical method are crucial in the success of any soil test. Published literature suggested rice roots may grow to a 60-cm depth in Arkansas and this knowledge was used to develop the initial soil sampling protocol used to evaluate levels of AH-N for rice production. Traditional soil sampling depths for rice (0–10 cm for P and K) would have resulted in significantly lower r^2 values and limited the predictive ability and applicability of AH-N for direct-seeded, delayed flood rice production for rice grown on silt loam soils in Arkansas. Lastly, sample to sample variability of the DSD is considerably lower than that of the ISNT and possibly the fractions quantified by the DSD are better indicators of potentially mineralizable N than the ISNT.

SUMMARY

The results presented here are promising and suggest that AH-N can be correlated and calibrated for crop response. Several key factors were identified that must be considered when evaluating a soil-based N test that quantifies potentially mineralizable-N. As previously stated use of AH-N in guiding fertilizer N rate decisions has the potential to increase the long-term sustainability of crop production while lowering the potential environmental impacts of excess N fertilization. However, proper steps must be taken to ensure that the methodology is evaluated within the proper confines (soil sampling depth, soil sample timing etc.) to promote its predictive ability and likelihood of success.

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