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Assessment of the Amino Sugar–Nitrogen Test on Iowa Soils: II. Field Correlation and Calibration

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ABSTRACT

There has been growing interest in using the amino sugar–nitrogen test (ASNT) to improve N fertilization of corn (*Zea mays* L.). The ASNT is intended to measure the soil organic N fraction that contributes to plant available N. The objectives of this study were to correlate the ASNT to corn N response measures and calibrate the test to Iowa soils and climatic conditions. Soil samples were collected in the fall, early spring, and late spring at the 0- to 15-cm and 0- to 30-cm sample depths. No significant correlation could be found between the ASNT and relative leaf chlorophyll meter value, relative grain protein, relative grain yield, grain yield response to applied N, and economic optimum N rate (EONR). The ASNT was not able to differentiate sites that were responsive or nonresponsive to N fertilization and could not be calibrated to EONR. There were strong linear correlations between the ASNT and total soil N (TSN), hydrolyzable NH_4–N, and hydrolyzable NH_4 + amino sugar–N. The ASNT was not significantly correlated to hydrolyzable amino sugar–N. The soils tested in this study had large amounts of hydrolyzable NH_4–N relative to hydrolyzable amino sugar–N, which may partially explain the poor results with the ASNT. Also, liberation of a constant proportion of TSN by the ASNT procedure explains the inability of the test to estimate a specific portion of soil N that contributes to plant available N. Based on the results of this work, the ASNT is not recommended in Iowa for estimating corn N responsiveness or adjusting N application rate.

The reported success with the amino sugar–nitrogen test (ASNT) by Khan et al. (2001) and Mulvaney (2006) has generated interest in the ASNT and its ability to improve N fertilization of corn. The ASNT, also referred to as the Illinois N soil test, is intended to measure the soil organic N fraction that contributes to plant available N during the growing season (Khan et al., 2001; Hoeft and Nafziger, 2002). However, a limited amount of research has been published regarding the correlation and calibration of the test to corn N response in the field.

In Illinois, Mulvaney et al. (2004) showed that the test can be successful in identifying soils that are nonresponsive to N fertilizer application. After completing over 100 small-plot N response trials, the ASNT correctly identified 90% of the sites where corn did not respond to N fertilization. Researchers in Arkansas evaluated the ASNT for N rate management in rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (Ross et al., 2005). Soil samples from the 0- to 10-cm soil depth were collected from N rate trials and analyzed for hydrolyzable amino sugar–N and the ASNT. Hydrolyzable amino sugar–N and the ASNT were correlated with N uptake and grain yield in both crops. Recent work in North Carolina evaluated several N soil tests in corn (Williams, 2005). When divided into soil classes (well and poorly drained), the ASNT had the highest correlation coefficient with economic optimum N rate (EONR) compared with other soil test methods evaluated in the study.

Osterhaus and Bundy (2005) evaluated the ASNT in Wisconsin using 81 N response experiments conducted from 1984 to 2004. The researchers found no relationship between the test and EONR, but there was a strong correlation between the ASNT and soil organic matter. In Michigan, Laboski (2004) conducted N rate experiments in corn during 2002 and 2003. The results showed a poor relationship between the ASNT and corn response to N fertilizer. Laboski (2004) concluded that more N rate trials were needed due to a lack of nonresponsive sites in the study. An experiment conducted by Torrie et al. (2004) in Saskatchewan, Canada also did not show a good correlation between ASNT values and N response in wheat. They concluded that the ASNT might be a reliable predictor of N response on soils with higher levels of soil organic matter.

Many proposed soil tests have measured the NH_3–N liberated directly from soil or soil extracts using NaOH (Cornfield, 1960; Keeney and Bremner, 1966a, 1966b; Geist and Hazard, 1975; Walmsley and Forde, 1976; Rojas, 1986; Wang et al., 2001). Some difficulties associated with these tests are high variability of results, a positive relationship with total soil N (TSN), a lack of correlation with N response measures in the field, and poor correlation with soil incubation results. Past N fractionation work has shown that determination of amino sugar–N was unsatisfactory due to the relatively small amount of amino sugar–N compared with TSN. Also, amino sugar–N is calculated by the difference from two separate distillations that measure hydrolyzable NH_4 + amino sugar–N and hydrolyzable NH_4–N (Ferguson and Sowden, 1966; Stevenson, 1996).

Nitrogen fertilizer recommendations in Iowa and several other states include soil NO_3–N testing in late spring to assess plant available soil N for corn (Magdoff et al., 1984; Blackmer et al., 1997). Use of this test is largely dependent on the producer applying N fertilizer in-season. However, most producers in Iowa apply N fertilizer in late fall or spring before planting. Developing a test that estimates potentially mineralized organic N from soil sampled before corn planting has the potential to enhance N fertilizer management. The objectives of this study were to evaluate the ability of the ASNT to

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Abbreviations: ASNT, amino sugar–nitrogen test; EONR, economic optimum nitrogen rate; LCM, leaf chlorophyll meter; TSN, total soil nitrogen; VT, tasseling corn growth stage.

estimate plant available N from soil by correlating the ASNT to corn N response measures and calibrating with EONR across Iowa soils and climatic conditions.

MATERIALS AND METHODS

Soil Sampling and Analysis

Nitrogen rate trials at 43 sites from 2001 to 2003 were used in this study and are described by Barker et al. (2006). The crop before the year studied at all sites was soybean [*Glycine max* (L.) Merr.]. Soil samples were collected from the 0- to 15-cm and 0- to 30-cm soil depths at random from each replication in October after soybean harvest before the crop year studied (fall) and in April before planting (early spring). Soil was also collected in June when corn was approximately 15 to 30 cm tall (late spring) at random from each no-N control plot at each site and from both soil depths. Samples were dried at 40°C in a forced-air oven and ground to pass through a 2-mm sieve.

Soil was analyzed for TSN, ASNT, hydrolyzable NH_4 + amino sugar–N, and hydrolyzable NH_4–N. Total soil N was determined by the Iowa State University Soil Testing Lab using the dry combustion method (Nelson and Sommers, 1996). The ASNT was performed on all soil samples in duplicate using direct soil diffusion techniques described by Khan et al. (2001) and Mulvaney (2006). Soil from one replicate at 11 selected N rate trial sites was analyzed for hydrolyzable NH_4 + amino sugar–N and hydrolyzable NH_4–N by the ^{15}N Analysis Service, Univ. of Illinois. Preparation of soil hydrolysates and the procedure used to determine hydrolyzable NH_4 + amino sugar–N and hydrolyzable NH_4–N was performed as described in Mulvaney and Khan (2001). Soil hydrolysates were prepared using a single fractionating procedure. Hydrolyzable NH_4 + amino sugar–N and hydrolyzable NH_4–N were analyzed from sep-

Table 1. Amino sugar–N test (ASNT) values at two soil sample depths and three sampling times and corn grain yield response from applied N at 43 N rate trials.

Site†	0- to 15-cm depth‡ Fall	0- to 15-cm depth‡ Early spring	0- to 15-cm depth‡ Late spring	0- to 30-cm depth‡ Fall	0- to 30-cm depth‡ Early spring	0- to 30-cm depth‡ Late spring	Yield with no N	Yield increase from applied N§
	mg kg^{-1}						Mg ha^{-1}	
				2001				
1	–¶	372	362	–	318	312	9.32	1.42
2	–	293	256	–	262	241	9.25	1.31
3	–	289	287	–	252	259	10.00	0.00
4	–	361	345	–	361	333	7.38	3.97
5	–	611	595	–	533	543	9.89	0.00
6	–	294	274	–	237	232	12.55	0.68
7	–	298	283	–	243	254	7.91	3.44
8	–	370	338	–	325	324	9.38	1.53
9	–	280	259	–	246	250	10.45	0.57
10	–	285	268	–	239	240	10.41	0.13
11	–	288	265	–	245	259	11.40	0.18
12	–	245	228	–	205	202	9.41	0.00
13	–	283	273	–	243	258	8.59	4.45
14	–	358	338	–	304	302	8.77	1.57
				2002				
15	295	287	297	250	256	252	13.88	0.00
16	249	245	274	262	241	245	8.62	2.50
17	314	298	324	293	305	298	10.74	1.06
18	329	319	332	300	320	316	10.89	1.65
19	358	351	368	326	316	302	8.59	2.55
20	430	423	435	384	387	356	10.05	1.77
21	279	271	310	265	271	282	10.91	2.65
22	298	292	308	284	269	267	13.59	0.00
23	272	296	–	282	264	248	3.29	0.91
24	242	253	242	257	250	250	10.52	1.37
25	245	232	247	264	268	263	11.35	3.65
				2003				
26	265	247	244	251	243	258	7.93	3.12
27	302	309	329	289	284	315	11.07	3.69
28	339	303	326	332	287	314	7.02	5.42
29	393	412	410	365	365	367	8.76	4.73
30	164	163	166	159	159	161	8.94	4.64
31	202	220	–	154	177	–	11.16	1.37
32	551	533	551	503	485	483	8.54	0.00
33	201	200	212	186	192	197	10.89	0.00
34	212	236	225	225	228	223	9.29	1.30
35	269	278	273	253	239	250	11.06	0.00
36	279	256	275	269	246	263	10.27	2.97
37	240	214	249	216	210	222	7.24	3.24
38	351	351	362	286	265	286	7.12	6.45
39	257	245	264	249	228	244	5.86	2.88
40	198	194	211	183	169	188	8.04	2.67
41	278	275	290	247	232	249	9.68	5.46
42	257	250	282	224	241	250	9.81	2.52
43	304	297	316	229	230	248	6.50	5.74

† Five of the study sites in 2001 (2, 5, 7, 9, and 12) were also study sites in 2003 (26, 32, 36, 39, and 40).
‡ Soil samples were analyzed in duplicate and are the mean of four replicates.
§ Yield at economic optimum N rate minus yield with no N.
¶ Samples not collected.

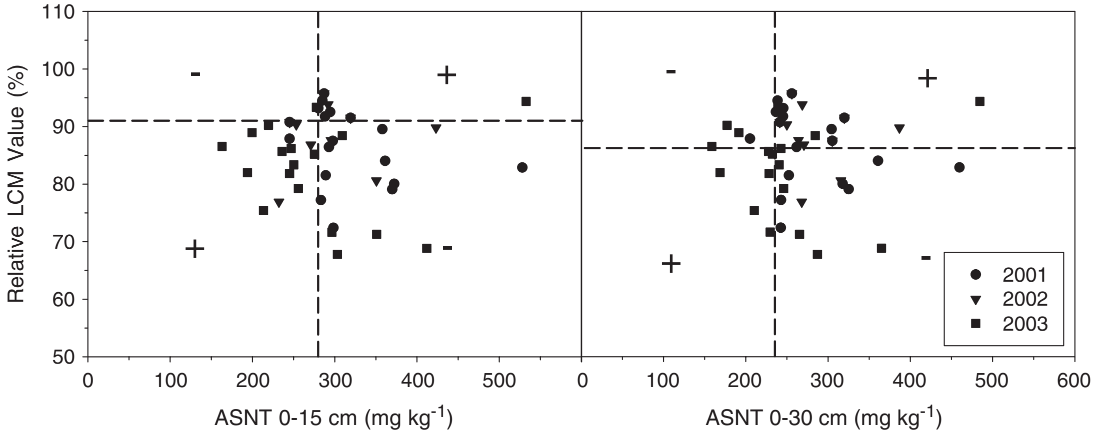

Fig. 1. Correlation of relative leaf chlorophyll meter (LCM) values at the tasseling stage (VT) and the amino sugar–N test (ASNT) from soil collected in early spring at the 0- to 15-cm and 0- to 30-cm depths at 43 N rate trials. When nearly all of the sites lie in the positive quadrants, the soil test is considered accurate in predicting response to added N fertilizer.

arate diffusion determinations. Hydrolyzable amino sugar–N was calculated by subtracting hydrolyzable NH_4–N from hydrolyzable NH_4 + amino sugar–N.

Corn Nitrogen Response Measures

Leaf chlorophyll meter readings were recorded to monitor the N status of corn plants at the tasseling corn growth stage (VT) (Ritchie et al., 1993). A Minolta SPAD-502 chlorophyll meter (Konica Minolta, Ramsey, NJ) was used to measure leaf greenness of the ear leaf from 25 corn plants from each N rate plot at all sites (Peterson et al., 1993). Relative leaf chlorophyll meter (LCM) values were calculated by dividing LCM readings from the no-N control by LCM readings from the highest N rate, multiplied by 100. Relative LCM values below 97% are an indication of corn N stress or deficiency (Sawyer et al., 2004).

Corn grain was hand harvested from the middle two rows (7.6-m length) of each plot after corn reached physiologic maturity. All grain yields were adjusted to 155 g kg^{-1} moisture content. Relative grain yields for each site were calculated by dividing grain yield of the no-N control by maximal yield determined from a regression model fit to yield response at each site, multiplied by 100. Economic optimum N rate was determined from the same regression model and calculated at the 10:1 corn-to-N fertilizer price ratio. Grain yield increase from applied N was calculated by subtracting the no-N control yield from the grain yield at EONR.

A subsample of corn grain was collected at harvest and analyzed for grain protein concentration by the Iowa State University Grain Quality Laboratory using near-infrared spectroscopy (Rippke et al., 1995). Relative protein concentration was calculated by dividing grain protein concentration from the no-N control by grain protein concentration from the highest N rate, multiplied by 100.

Statistical Analysis

The SAS system version 8.2 was used for statistical analyses of all data (SAS Institute, 2001). Yield response to applied N at each site was analyzed in a step-wise process. First, significance of N rate was determined using PROC GLM as main effect of N rate or contrast of no-N vs. applied N. If not significant ($p > 0.10$), the site was classified as nonresponsive to applied N. If significant, regression models were fitted using PROC NLIN. If the models had a similar R^2 value and had a significant fit ($p < 0.10$), then quadratic-plateau or quadratic equations were selected in preference to linear-plateau or linear models. Otherwise, the model with a significant fit and largest R^2 value was chosen. The response fit was also visually inspected against yield at each N rate to confirm the appropriate choice of model.

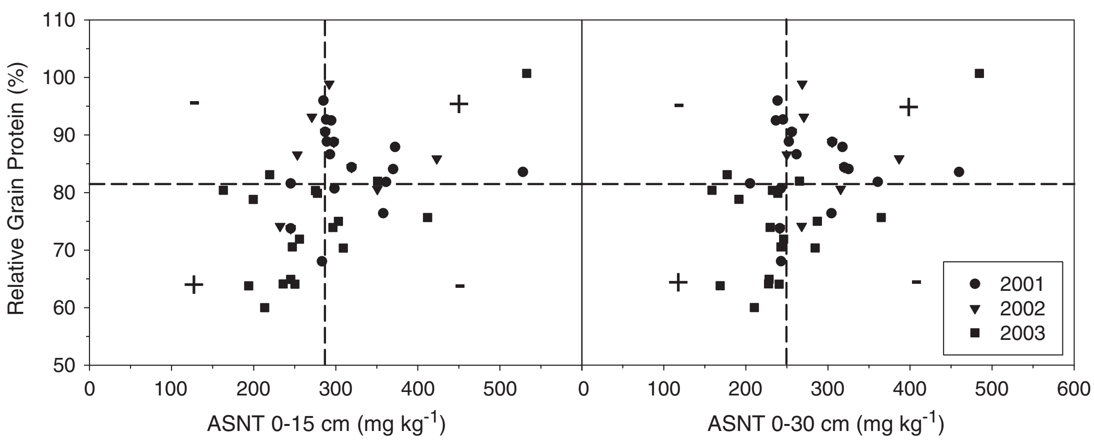

Fig. 2. Correlation of relative grain protein and the amino sugar–N test (ASNT) from soil collected in early spring at the 0- to 15-cm and 0- to 30-cm depths at 43 N rate trials. When nearly all of the sites lie in the positive quadrants, the soil test is considered accurate in predicting response to added N fertilizer.

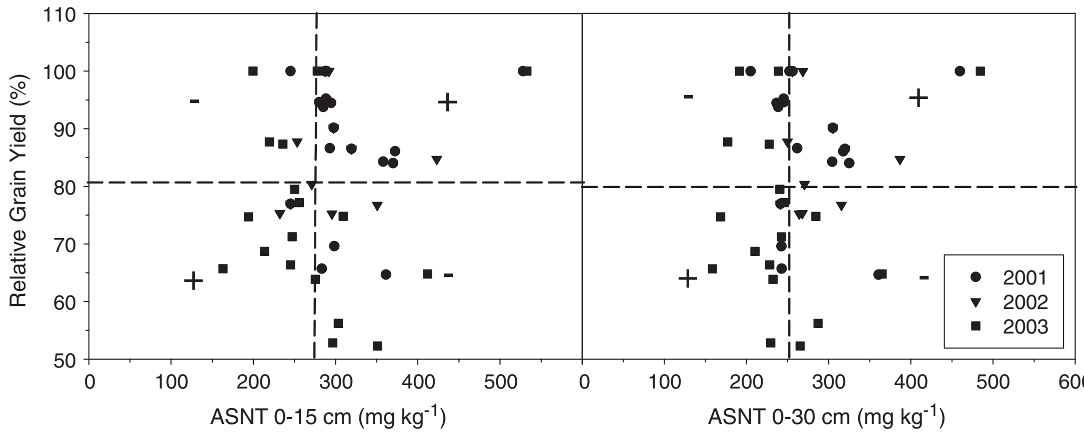

Fig. 3. Correlation of relative grain yield and the amino sugar–N test (ASNT) from soil collected in early spring at the 0- to 15-cm and 0- to 30-cm depths at 43 N rate trials. When nearly all of the sites lie in the positive quadrants, the soil test is considered accurate in predicting response to added N fertilizer.

The Cate-Nelson graphical method (Dahnke and Olson, 1990) was used to correlate the ASNT to corn N response measures and separate sites into two populations of responsive and nonresponsive to applied N. A clear plastic overlay with two intersecting perpendicular lines (upper right and lower left quadrants labeled +, upper left and lower right quadrants labeled −) was positioned over scatter diagrams. The overlays were positioned on the scatter diagram vertically and horizontally until the maximum number of sites was located in the positive quadrants. When nearly all of the sites lie in the positive quadrants, the soil test is considered accurate in predicting response to added N fertilizer. A statistical procedure (Cate and Nelson, 1971) for separating sites into two populations (responsive and nonresponsive to applied N) was also performed by using successive ASNT concentrations to determine a critical concentration that maximizes the R^2 value.

A linear regression model was used to compare the ASNT with grain yield increase to applied N, EONR, TSN, hydrolyzable NH_4 + amino sugar–N, hydrolyzable NH_4–N, and hydrolyzable amino sugar–N.

RESULTS

Correlation with Corn Nitrogen Response

The ASNT values from soil samples collected in fall, early spring, and late spring at the 0- to 15-cm and 0- to 30-cm depths (Table 1) were evaluated for relationship to corn N response. The ASNT values (Barker et al., 2006) and correlations to N response found for each soil sampling time were similar; therefore, results from only early spring are presented and discussed. Correlation of the ASNT to N response with sites categorized into subgroups based on physical and chemical soil properties was also attempted, but there was no improvement in the ASNT correlation.

Figure 1 shows the relationship between ASNT values at two soil sample depths and relative LCM values at the VT growth stage. The ASNT did not correlate well with LCM values at the 0- to 15-cm depth ($R^2 = 0.14$) or the 0- to 30-cm depth ($R^2 = 0.03$). There were many sites in the lower negative quadrant from each year that had large ASNT values, but corn plants showed signs of N deficiency. The ASNT was not able to distinguish between sites showing N deficiency and sites with adequate N at the VT growth stage.

Figure 2 presents a comparison between the ASNT at the two sample depths and relative protein concentration in harvested grain, where grain protein can reflect season-long plant response to N (Pierre et al., 1977). The correlation between the ASNT and relative grain protein was similar to the ASNT relationship with LCM values measured earlier in the growing season. The R^2 values for the 0- to 15-cm and 0- to 30-cm depths were 0.33 and 0.21, respectively. Again, many sites were located in the upper and lower negative quadrants for both soil sample depths. The test was unable to separate sites into responsive and nonresponsive groups based on grain protein concentration.

There was also a poor correlation between the ASNT at each soil sample depth and relative grain yield (R^2 = 0.07 and 0.00 at the 0- to 15-cm and 0- to 30-cm depths, respectively) (Fig. 3). This demonstrates that the ASNT was not able to differentiate grain yield response to

Table 2. Regression models for the relationship between grain yield increase from applied N and economic optimum N rate (EONR) with the amino sugar-N test (ASNT).

Relationship	Soil sample depth	Year	Regression model†	n§	R^2¶
	cm				
Grain yield increase	0–15	2001	$y = 1.6 - 0.001x$	14	0.01
		2002	$y = 2.7 - 0.003x$	11	0.03
		2003	$y = 2.9 + 0.001x$	18	0.01
	0–30	2001	$y = 1.0 + 0.001x$	14	0.01
		2002	$y = 1.0 + 0.002x$	11	0.01
		2003	$y = 3.6 - 0.002x$	18	0.01
			Regression model‡		
EONR	0–15	2001	$y = 59 + 0.027x$	14	0.01
		2002	$y = 128 - 0.146x$	11	0.03
		2003	$y = 140 - 0.102x$	18	0.02
	0–30	2001	$y = 33 + 0.120x$	14	0.02
		2002	$y = 70 + 0.049x$	11	0.01
		2003	$y = 158 - 0.188x$	18	0.06

† *x*, ASNT (mg kg^{-1}); *y*, grain yield increase to applied N (Mg ha^{-1}).
‡ *x*, ASNT (mg kg^{-1}); *y*, EONR (kg N ha^{-1}).
§ *n*, number of sites.
¶ All regression models were not statistically significant at the 0.05 probability level.

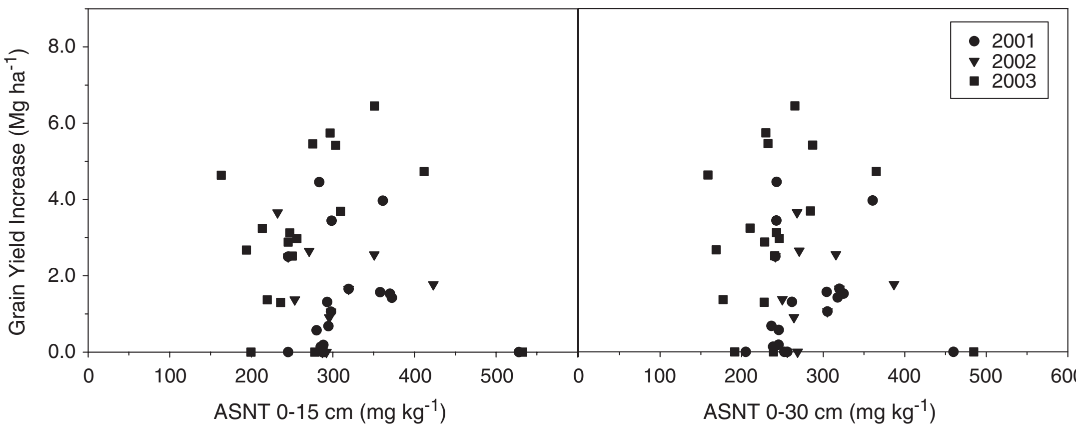

Fig. 4. Correlation of grain yield increase from applied N and the amino sugar–N test (ASNT) from soil collected in early spring at the 0- to 15-cm and 0- to 30-cm depths at 43 N rate trials.

applied N across the Iowa soils studied. The lower negative quadrant had a high percentage of sites with both soil sample depths but had relatively low grain yields. A large number of sites with relative yields ranging from 50 to 100% had similar ASNT values. This distribution makes it impossible to separate sites that are responsive and nonresponsive to N fertilizer application or to differentiate N response level.

An attempt was made to correlate the ASNT at each soil sample depth with grain yield increase to N application (Table 2, Fig. 4). The linear correlation for each soil sample depth was never significant between ASNT value and yield increase from applied N. There was no distinct separation of responsive and nonresponsive sites. A number of sites that were responsive to N application had high ASNT values. Also, many of the nonresponsive sites had ASNT values too low to improve the correlation relationship.

There was no relationship between ASNT values and EONR (Table 2, Fig. 5). Economic optimum N rates ranged from 0 to 200 kg N ha^{-1} at relatively similar ASNT values for both soil sample depths, so there was also no differentiation of sites with an EONR of 0 kg N ha^{-1} and 200 kg N ha^{-1}. Amino sugar–N test concentrations for nonresponsive sites ranged from less than 200 mg kg^{-1} to greater than 450 mg kg^{-1}.

DISCUSSION

The results of this study are similar to other field correlation research with the ASNT in corn and wheat (Laboski, 2004; Torrie et al., 2004; Osterhaus and Bundy, 2005). The lack of correlation with N response measures and inability to be calibrated with EONR clearly indicates the ASNT is not useful for making N rate recommendations. Similar N soil tests using NaOH to liberate NH_3–N have not been well correlated to N response measures or soil incubation experiments (Keeney and Bremner, 1966a, 1966b; Walmsley and Forde, 1976; Wang et al., 2001). The lack of correlation between the ASNT and corn N response measures in this study may be explained by a number of factors.

The original development of the ASNT assumed that normal weather conditions existed during the growing season (Khan et al., 2001; Mulvaney, 2006). The wide range of climatic conditions between sites and years during this study may have varied N mineralization potentials and crop N need to a degree that the N test was unable to give an accurate prediction of N response. Kresge and

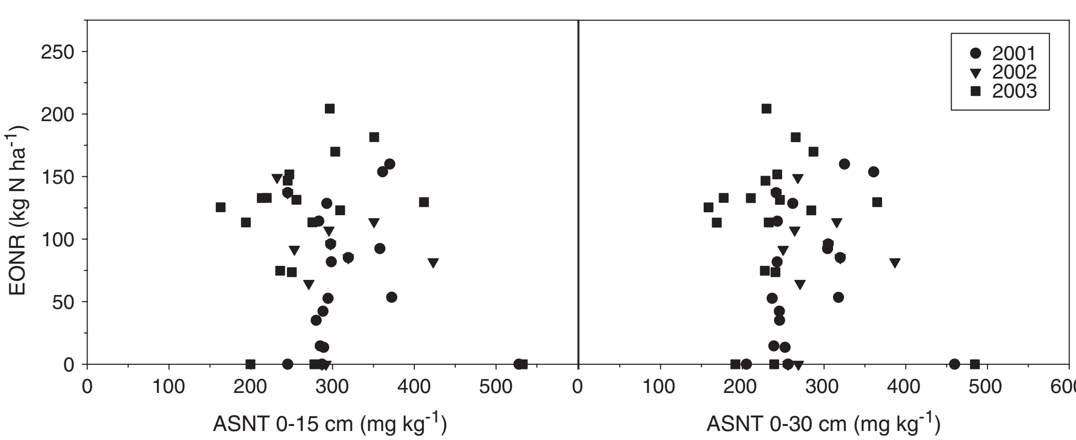

Fig. 5. Calibration of economic optimum N rate (EONR) and the amino sugar–N test (ASNT) from soil collected in early spring at the 0- to 15-cm and 0- to 30-cm depths at 43 N rate trials.

Merkle (1957) evaluated N soil test methods that determined alkali distillable N and found the method of questionable value when determining crop N fertilizer requirements for the current year. They concluded the conditions that control nitrification and nitrate utilization by the crop were more important than the quality of existing N in the soil. Perhaps future soil N test work should include field calibration using N response from multiple years at the same sites to account for climatic variation across different soils.

Past alkali distillation procedures have shown a strong relationship with TSN (Keeney, 1965; Geist and Hazard, 1975; Rojas, 1986). Typically, TSN has been considered a poor indicator of N fertilizer responsiveness in corn. There was a positive correlation between the ASNT and TSN in this study (Fig. 6). Total soil N in samples collected in early spring at the 0- to 30-cm depth had a significant linear relationship to ASNT values. The linear fit varied somewhat between years. When averaged across all sites, approximately 15% of TSN was liberated by the ASNT procedure. The strong relationship between TSN and the ASNT indicates that the ASNT is likely not selectively reflecting the amount of amino sugar–N in the soil.

Khan et al. (2001) evaluated N rate trials that included some soils containing substantial amounts of fixed, nonexchangeable NH_4–N and speculated that ASNT values would increase if fixed NH_4–N were liberated by the proposed test. The ASNT also includes exchangeable NH_4–N. In this study, the amount of exchangeable NH_4–N in soils at each of the sites was between 7 and 24 mg kg^{-1} (Barker et al., 2006). Hydrolyzable-N was analyzed at 11 selected N rate trial sites from soil collected in early spring at the 0- to 30-cm depth (Fig. 7). A strong, statistically significant linear correlation existed between hydrolyzable NH_4 + amino sugar–N and the ASNT. The hydrolyzable NH_4–N fraction was large in these soils and was much greater than the amino sugar–N fraction. There was also a strong linear correlation that was statistically significant between hydrolyzable NH_4–N and ASNT. The ASNT was not significantly correlated with hydrolyzable

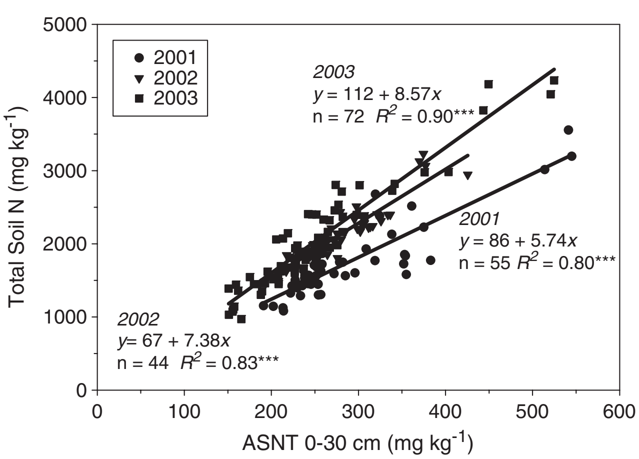

Fig. 6. Relationship of total soil N (TSN) and the amino sugar–N test (ASNT) from soil samples collected in early spring at the 0- to 30-cm depth from each replication at 43 N rate trials. ***Statistically significant at the 0.001 probability level.

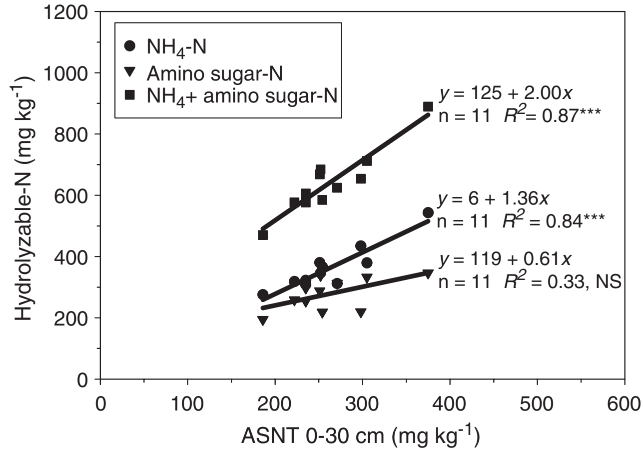

Fig. 7. Relationship of hydrolyzable-N and the amino sugar–N test (ASNT) from soil samples collected in early spring at the 0- to 30-cm depth from one replication at each of 11 N rate trials (sites 3, 7, 10, 11, 13, 15, 16, 18, 20, 22, and 25). ***Statistically significant at the 0.001 probability level; NS, not statistically significant at the 0.05 probability level.

amino sugar–N. This indicates that the ASNT is a good estimate of the hydrolyzable NH_4–N fraction but is not for amino sugar–N. As a result, the ASNT does not accurately measure amino sugar–N levels in the soil but instead reflects the larger hydrolyzable NH_4–N fraction combined with the amino sugar–N fraction. Stevenson (1996) previously noted limitations of the alkali decomposition method for determining hydrolyzable amino sugar–N. The method was considered unsuitable for soils containing low concentrations of amino sugar–N, especially when a high concentration of hydrolyzable NH_4–N was present.

CONCLUSIONS

There were no positive correlations between the ASNT and corn N responses, relative yield, yield response to applied N, or EONR across the soils and climatic conditions studied. The ASNT was also not able to differentiate sites into categories of responsive and nonresponsive to applied N. Therefore, the ASNT is not helpful for adjusting corn N fertilization rate. The reason for this lack of correlation may be explained by the highly significant linear relationships between TSN and ASNT values, hydrolyzable NH_4–N and ASNT values, and the lack of relation between ASNT values and hydrolyzable amino sugar–N in the soils tested. Iowa soils may have a hydrolyzable NH_4–N fraction that is too large for the ASNT to provide an accurate measurement of amino sugar–N. Based on this research, use of the ASNT is not recommended in Iowa for estimating N responsiveness or adjusting corn N fertilization.

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