^{13}C DISCRIMINATION AND REMOTE SENSING CAN BE USED TO EVALUATE SOYBEAN YIELD VARIABILITY

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ABSTRACT

Diagnostic tools for measuring yield variability and assessing the cause of soybean (*Glycine max*) yield variability are needed to evaluate the impact of different management options on profitability. The objectives of this study were to determine if remote sensing and ^{13}C discrimination (Δ) can be used to evaluate soybean yield variability. Research was conducted in five eastern South Dakota fields between 1999 and 2001. At 50 sampling points in three fields (Brookings, Moody, and SDSU): (i) crop reflectance was measured; (ii) gravimetric soil water (0-60 cm) content was measured periodically during the growing season; (iii) soybean yields were measured by a combine equipped with a yield monitor and differentially corrected global positioning system (DGPS); and (iv) plant samples were collected and analyzed for total N and Δ. Elevation and sampling point locations were measured with a carrier phase DGPS. Crop reflectance was used to calculate the green normalized differential vegetation index (GDVI=(near infrared -green)/(near infrared+Green)] and the red normalized differential vegetation index (NDVI= (near infrared-red)/(near infrared+red)]. In Brookings, Moody, SDSU, and Lovjoy soybean yield was positively correlated to GDVI, NDVI, and NIR (near infrared) reflectance measured in August. Remote sensing by itself could not be used to identify the factors responsible for yield variability. Based on soil water and), that were lower in summit and shoulder areas than footslope areas, reduced yields in summit and shoulder areas were attributed to water stress. Results from this experiment suggest that) can be used to assess the impact of water stress in soybean.

KEYWORDS: drought, water use efficiency, spatial variability

INTRODUCTION

To improve management decisions, techniques for measuring and identifying the cause of yield variability in production fields are needed. In the Great Plains either too little or too much water are primary factors causing soybean yield variability. In semi-arid environments, evapotranspiration (ET) can be estimated using a mass balance approach (Hatfield et al., 2001). However, unless runoff and runin are considered in fields containing topographic relief, these estimates may not be accurate. To solve this problem, waterflow models have been used to estimate runoff and runin. Water flow models linked to crop growth models can be used to determine yield losses due to water stress (Batchelor and Paz, 1999; Basso, 2000;). However, to calibrate a model such as CROPGRO-soybean, yield information from a number of different sites and years are needed. This information is not available for most production fields. Remote sensing has been proposed as a tool to estimate crop yields (Bauer, 1985; Moran et al., 1997; Ma et al., 2001). However, because many factors (photosynthesis, evaporation, and plant growth) interact to influence crop yield and reflectance, it is possible that water and a variety of nutrient stresses can increase reflectance in the green band (500-600 nm) and reduce reflectance in the near infrared (NIR; 700-900 nm) band (Blackmer et al., 1996; Moran et al., 1997; Inoue et al., 1998). Therefore, to resolve the factor or factors responsible for yield variability, ground collected information may be needed.

Most C_3 plants, including soybean, respond to water stress by closing the stomata, which reduces photosynthesis, leaf size, leaf area index, biomass production, seed size and numbers, and accelerate leaf senescence (Souza et al., 1998). However, in legumes such as soybean, water stress can also reduce N_2 fixation (Serraj and Sinclair, 1996; Serraj et al., 1998), and therefore, in landscapes with variable levels of available water, it is possible that water stress can reduce yields by two separate mechanisms (N stress caused by reduced N fixation and water stress resulting in stomatal closure and reduced photosynthesis). Water and N stress have opposite effects on isotopic ^{13}C discrimination (Δ) (Clay et al., 2001a; Clay et al., 2001b). Prior to discussing why N and water stress have opposite effects on Δ a few definitions are needed.

The ratio between ^{13}C and ^{12}C is the R value (O'Leary, 1993). The R value is used to calculate $*^{13}C$ using the equation:

$$*^{13}C = [R(sample)/R(standard)-1] \times 1000‰ \qquad (1)$$

where R(sample) is the $^{13}C/^{12}C$ ratio of the sample and R(standard) is the $^{13}C/^{12}C$ ratio of PDB, a limestone from the Pee Dee formation in South Carolina (O'Leary, 1993; Farquhar and Lloyd, 1993). Typically, $*^{13}C$ values for air, C3, and C4 plants are -8, -27, and -13‰, respectively. A negative sign indicates that the sample has a lower $^{13}C/^{12}C$ ratio than PDB. In many cases, it is convenient to report ^{13}C discrimination ( )), which is calculated using the equation:

$$) = (*^{13}C_a - *^{13}C_p)/(1 + *^{13}C_p/1000) \qquad (2)$$

where $*^{13}C_a$ is the $*^{13}C$ value of air(-8‰) and $*^{13}C_p$ is the measured value of the plant.

Isotopic) can be used to evaluate water stress in C_3 plants because ribulose bisphosphate carboxylase (RuBisCO), which catalyzes the combination of CO_2 with ribulose diphosphate to form two molecules of 3-phosphoglyceric acid, discriminates against $^{13}CO_2$ (O'Leary, 1993; Farquhar and Lloyd, 1993). If the plants are not water stressed, then stomata are open and discrimination is high. However, if plants are water stressed, then the stomata are partially closed, which in turn reduces). In C_3 plants, photosynthesis induced ^{13}C discrimination ( )) has been described by the equation:

$$)_{C3} = a + (b-a)C_i/C_a \qquad (3)$$

where, a is the ^{13}C discrimination due to CO_2 diffusion in air (4.4‰), b is ^{13}C discrimination caused by carboxylation (30‰, when corrected for the equilibrium effect of CO_2 dissolution), C_i is the intercellular partial pressure of CO_2, and C_a is atmospheric CO_2 partial pressure (O'Leary, 1993; Farquhar and Lloyd, 1993). Equation 3 predicts that as C_i/C_a goes to 0 (stomata closed), $)_{C3}$ goes to 4.4 and as C_i/C_a goes to 1 (stomata open), $)_{C3}$ goes to 30. In other words, equation 3 predicts that) decreases with increasing water stress

Many plants respond to water stress by closing the stomata and to N deficient conditions by producing less chlorophyll. Under these conditions, equation 3 predicts that in C_3 plants N stress will increase Δ and water stress will decrease Δ. The hypothetical impact of water and N stress on Δ in C_3 and C_4 plants were confirmed in field studies conducted by Clay et al. (2001a), Clay et al. (2001b), and Smeltekop et al. (2002). In a Montana study , Clay et al. (2001b) reported that in wheat (*Triticum aestivum* L.), a yield loss of 1 Mg ha^{-1} due to water stress resulted in a 1.131‰ decrease in Δ, and that under N limited conditions, N stress increased Δ by 0.01‰ for every kg of N that the plant was N deficient. Smeltekop et al. (2002) and Clay et al. (2001a) developed approaches for using) combined with the plants N percentage to determine yield losses due to N and water stress in corn (*Zea mays* L.) and wheat. Similar experimental approaches for evaluating yield limiting factors in soybean are needed. The objectives of this study were to determine if remote sensing and Δ can be used to evaluate soybean yield variability.

MATERIALS AND METHODS

Research was conducted in five fields (Brookings in 1999, Moody in 2000, SDSU 2001, Lovjoy 2000, and TE80 2000) located in eastern South Dakota. The field designations, soil types, latitude and longitude coordinates, crop varieties, tillage, rotations, and plant populations of each field are reported in Table 1. In Brookings and Moody, soil samples from each soil horizon in the dominant soil series were collected in 2001. Soil bulk density and the water content at the permanent wilting point (1.5 Mpa) were determined on these samples (Klute, 1986). Elevation at all sampling sites was measured with a carrier-phase differentially corrected global positioning system (DGPS). A weather station located within 15 km of each site was used to measure precipitation and air temperature. Growing degree days were calculated using a base 10^o C. In the year prior to planting soybean, soil samples (minimum eight cores per composite sample) were collected from the 0 to15 cm depth from at least a 1 ha grid. These samples were analyzed for Olsen P and K (Frank et al., 1998; Warnche and Brown, 1998). Based on the laboratory analysis, P and K fertilizers were applied for the two-year rotation. Weeds were controlled with appropriate herbicides. Yields at all fields were measured with a calibrated yield monitor (Lems et al., 2001).

At Brookings in 1999, gravimetric water contents at two depths (0-15 and 15-60 cm) were determined on soil samples collected from 50 points located on 4 transects on 13 July, 27 July, 4 August, 17 August, and 26 August. Whole plant samples harvested from a 1 m^2 area near each sampling point were collected on 13 July, 27 July, 10 August, and 26 August. Plant samples were dried, ground, and analyzed for total N and Δ. Four bands (557-582 nm, green; 602-627nm, yellow; 647-672 nm, red; and 720-920 nm , NIR) digital aerial images (0 to 255 brightness values) with 1 m pixel resolution were collected on 27 July. Digital information was used to

Table 1. Field locations, dominant soil types, and landscape type at the different study sites.

	Field identifier				
	Brookings	Moody	SDSU	Lovjoy	TE80
Study year	1999	2000	2001	2000	2000
Latitude(^{o}N)	44.23	44.17	44.35	44.25	44.06
Longitude(^{0}W)	96.65	96.62	96.81	96.93	96.63
Rainfall (May-Oct.) cm	42.2	40.8	42.6	35.7	40.8
Growing degree days	1365	1308	1361	1355	1308
Dominant soil types	Barnes Calcic Hapludoll	Cubden Aeric Calciaquoll	Barnes Calic Hapludoll	Hamerly Aeric Calciaquoll	Moody Udic Haplustoll
	Brookings Aquic Calciaquoll	Waubay Pachic Hapludoll	Svea Pachic Hapludoll	Barnes Calcic Hapludoll	Houdek Typic Argiustoll
	McIntosh Aeric Calciaquall	Kranzburg Calcic Hapludoll		Vienna Calcic Hapludoll	Lamo Cumulic Endoaquoll
	Vienna Calcic Hapludoll	Vienna Calcic Hapludoll			
Tillage	Strip tillage	Strip tillage	Chisel	Strip tillage	Chisel
Rotation	Soybean/corn	Soybean/corn	Soybean/corn	Soybean/corn	Soybean/corn
Soybean variety	Pioneer 91B91	Pioneer 92B05	Zillers 1750	Pioneer 91B91	Garst 198 RR
Population (plants ha^{-1})	395,000	371,000	317,000	371,000	519,000
Fertilizer applied	At all sites N, P, and K fertilizer applied to corn				

calculate the green normalized differential vegetation index (GDVI = (NIR-green)/(NIR+Green)) and the red normalized difference vegetation index (NDVI=(NIR-red)/(NIR+Red)). Based on the known locations of 4 tarps, aerial images were georeferenced. The locations of the sampling points were overlaid onto the digital remote sensed image and yield map. The pixel values and yields (0% moisture and 83.6 m^2 area) were determined.

At Moody in 2000, gravimetric soil water contents were measured on soil samples (0-15 and 15-60 cm) collected from 50 sampling points on 7 June, 28 June, 18 July, 5 September, and 27 September. Soil samples from individual horizons at sampling points in the divergent summit and shoulder and convergent footslope areas were analyzed for plant available water. Four band digital aerial images with 1m pixel resolution were collected on 28 July and 14 August. Based on the known locations of 6 tarps, aerial images were georeferenced. The locations of the sampling points were overlaid onto the digital image and the pixel values at the sampling points were determined. At these same sampling points, grain samples were hand collected on 27 September from a 1 m^2 area.

In Moody in 2000, a randomized block experiment was conducted to determine the impact of water stress and landscape position on yield and). The experiment, conducted in the divergent summit/shoulder and convergent footslope positions, contained two treatments (plants that were and were not watered weekly with 3.81 cm of water). The experiment had 8 blocks at each landscape position. In each watered plot, water was applied to a single 15 cm plastic ring pounded 7.5 cm into the ground. The watered plants were within 15 cm of the ring. Soybean plants were hand harvested at physiological maturity. The ring was used to insure that water infiltrated into the soil. Soybean plants located within 15 cm of the ring were harvested for the watered treatments. For the non-watered treatments, plants from non-watered adjacent areas were harvested.

In a field located near South Dakota State University (SDSU), gravimetric water contents of the 0-15 and 15-60 cm were measured on 21 June, 12 July, 26 July, and 6 August 2001 at over 50 points. Crop reflectance in the blue, green, red, and NIR wavelength regions were measured by the IKONOS satellite on 25 July. The imagery had a pixel resolution of 4 by 4 m. Remote sensed information was used to calculate GDVI and NDVI using equations described above. At maturity, a plot combine was used to measure soybean yields in a 1.52 by 6.1 m area for each sampling point. A randomized block experiment, identical to the experiment described above, was conducted in this field to determine the impact of water stress and landscape position on yield and). The experiment, conducted in the divergent summit/shoulder and convergent footslope positions, contained two treatments (plants that were and were not watered weekly with 3.81 cm of water). The experiment had 4 blocks at each landscape position.

At Lovjoy in 2000, grain samples were hand collected at maturity from a 1 m^2 area at 45 sampling points within the field. A handheld multispectral radiometer (MRS16, Cropscan, inc, Rochester, MN) measured relative crop reflectance at 560, 568, 610, 660, 710, 760, 810, 830, 905, and 1050 nm (Ma et al., 2001) on 14 August. Each band had a half peak band width of between 5 and 15 nm. The sensor head was mounted on an adjustable pole that was parallel to the ground surface with a field of view of between 0.8 to 1.0 m diameter. NDVI and GDVI were calculated as described above.

At the TE80 field grain samples were collected after maturity from 18 points in 2000. These sampling points were located at shoulder, backslope, and footslope positions. Elevations at these points were measured with a DGPS. At TE80 crop reflectance was not measured.

All grain and whole plant samples were analyzed for total N, *^{13}C, and Δ on a 20-20 Europa ratio mass spectrometer ((Europa Scientific Ltd., UK). Grain samples from Moody , SDSU, and Lovjoy were analyzed for oil and protein on a NIR S5000 Foss Tech (Silver Spring, MD).

RESULTS AND DISCUSSION

Yield Spatial Variability

Site characteristics

During the season precipitation (May through October) ranged from 35.7 at Lovjoy to 42.6 cm at SDSU. Growing degree days ranged from 1300 GDD at Moody to 1365 GDD at Brookings (Table 1). Each site had gently undulating to gently rolling topographies and contained both well (Brookings, Vienna, and Moody) and poorly drained soils (McIntosh, Cubden, Hamerly, and Lamo). Parent materials were loess over glacial till or alluvium. Surface pH values in summit/shoulder soils ranged from 6 to 7 and the surface pH values in footslope soils ranged from 7 to 8.

Table 2. The mean, standard deviation, medium, and skewness for yield and C^{13} discrimination at the 5 study sites.

Field	Mean	Standard deviation	Median	Skewness
		Grain yield (kg ha^{-1})		
Brookings	2520	292	2650	-2.25
Moody	2460	354	2390	0.41
SDSU	2450	576	2600	-0.32
Lovjoy	2120	585	2151	-0.05
TE80	2620	196	2630	0.21
		C^{13} discrimination (‰)		
Brookings				
13 July	20.1	0.41	20.1	-2.00
10 August	20.0	0.43	20.1	-1.40
26 August	19.5	0.60	19.5	-0.76
Moody (harvest)	19.4	0.56	19.5	-0.26
SDSU				
20 July	19.85	0.44	19.89	-0.15
16 August	19.44	0.75	19.64	-0.76
Harvest	19.12	19.73	19.27	-0.78
Lovjoy (harvest)	18.7	0.73	18.5	0.52
TE80 (harvest)	19.7	0.28	19.7	-0.05

Spatial yield variability

Grain yields were greatest at TE80 and least at Lovjoy (Table 2). TE80 also had the lowest CV (CV=Stardard deviation/mean), while Lovjoy had the highest CV. In four fields (Brookings, Moody, SDSU, and Lovjoy) yields were generally less in divergent summit/shoulder than convergent footslope positions.

At Brookings, yields in the high elevation areas were 30-50% less than yields in the low elevation areas (Fig. 1) and were positively correlated to GDVI, NDVI, and NIR reflectance (Table 3).

At Moody, soybean yields in high elevation (divergent summits/shoulder) areas were 20 to 50% less than yields in low elevation areas (Fig. 1). A yield map overlaid on a topographic map was used to demonstrate the extent of yield variability observed in the fields (Fig. 2). The other fields had similar spatial relationships. Yield was positively correlated to NDVI measured on 28 July and negatively correlated to elevation (Table 3). In digital images collected in August, yield was positively correlated to GDVI, NDVI, and NIR. Differences in the correlation coefficients between yield and remote sensing on 28 July and 14 August demonstrate the temporal nature of remote sensing. Similar results were observed at SDSU and Lovjoy (Fig. 1, Table 3). At both of these sites, yield was positively correlated to GDVI, NDVI, and NIR; and yield was negatively correlated to elevation.

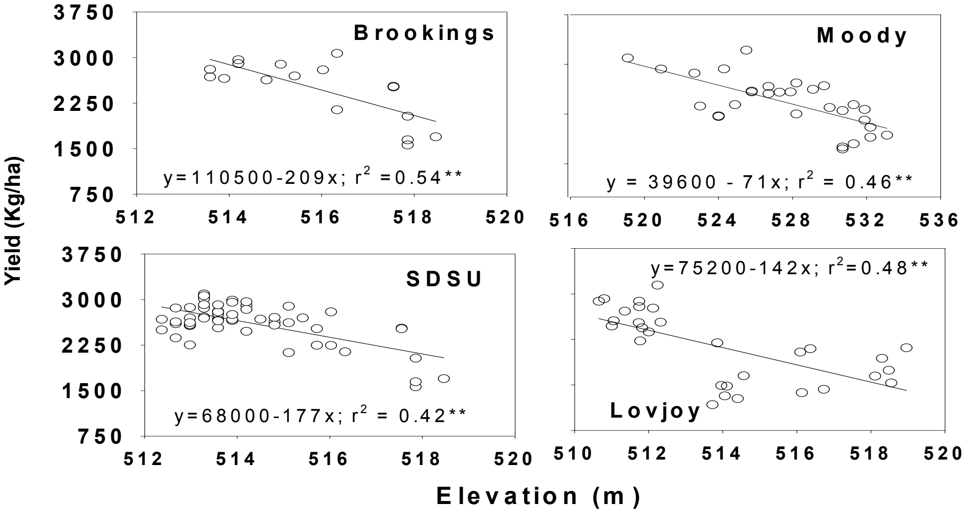

Figure 1. The relationships between yield and elevation at Brookings, Moody, SDSU, and Lovjoy.

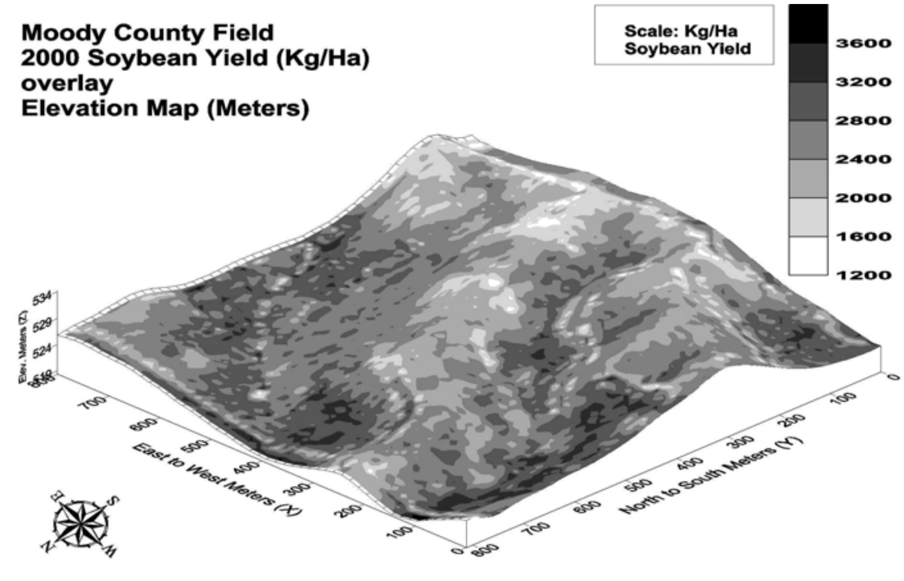

Figure 2. The Moody yield map superimposed on an elevation map.

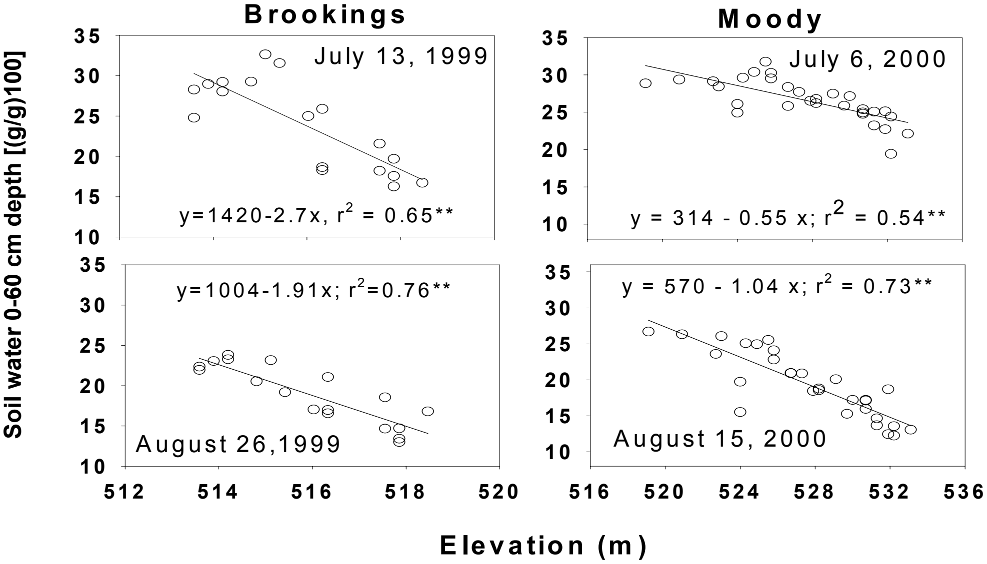

Figure 3. The relationships between soil water and elevation at several dates in the Brookings and Moody fields.

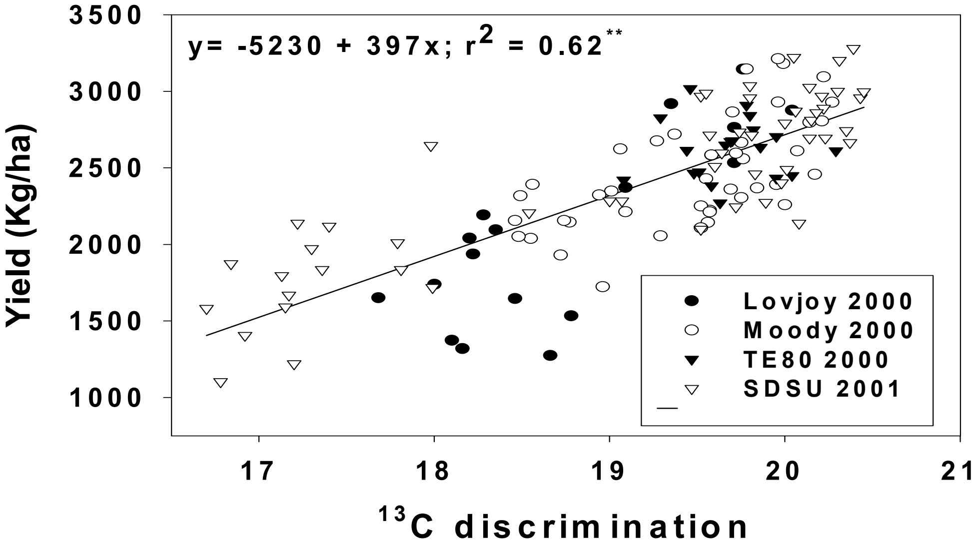

Figure 4. The combined relationship between yield and ^{13}C discrimination ( )) at The Moody, Lovjoy, TE80, and SDSU fields.

Table 3. Correlation coefficients between yield and remote sensing and elevation data collected at 4 sites.

	Remote sensing							Significant r	
Field	collected	GDVI	NDVI	Green	Red	NIR	Elevation	0.05	0.01
		--------------------r----------------------------							
Brookings	Aug.	0.32	0.44	-0.14	-0.29	0.56	-0.42	0.273	0.354
Moody	July	0.21	0.28	0.23	0.15	0.26	-0.57	0.273	0.354
Moody	Aug.	0.51	0.47	-0.48	-0.47	0.40	-0.57	0.273	0.354
SDSU	Aug.	0.56	0.52	0.17	0.17	0.55	-0.68	0.273	0.354
Lovjoy	Aug.	0.89	0.86	-0.70	-0.81	0.87	-0.69	0.335	0.456

Causes of yield variability

At Brookings and Moody, gravimetric soil water contents were influenced by landscape position and generally were lower in high than low elevation areas (Fig. 3). For example, at Brookings gravimetric soil water contents ranged from 0.15 g g^{-1} in the divergent summit/shoulder areas to over 0.30 g g^{-1} in the convergent footslope areas on July 13. As the season progressed, soils in the summit.shoulder area dried out faster than soils in the footslope position. At Moody in summit/shoulder areas, gravimetric water contents on 15 August approached 0.15 g g^{-1} soil, while in footslope areas water contents ranged from 0.2 to 0.27 g g^{-1} soil. Based on the gravimetric water contents and the permanent wilting point (1.5 Mpa) in summit/shoulder (0.13 g g^{-1} soil) and footslope (0.15 g g^{-1} soil) areas, the amount of plant available water on 15 August in summit/ shoulder and footslope areas (surface 60 cm of soil) were 1.6 and 7.8 cm, respectively. Similar results for spatial and temporal changes at SDSU were observed (Data not shown). Landscape differences in plant available water may have resulted from several factors. First, runoff from divergent summit/shoulder areas with subsequent runin into convergent footslope areas influenced the total available water. Second; capillary movement of water from groundwater to the rootzone in footslope areas increased available water. Third, lateral movement of water from summit/shoulder to footslope areas has the potential to increase available water.

Spatial and temporal variability in) was observed in all fields. At Brookings,) in whole plant samples collected on 27 July at Brookings ranged from 20 to 21‰. As the season progressed,) decreased faster for plants located in high elevation than low elevation areas (data not shown). Smedley et al. (1991) had a similar temporal response in), and attributed decreasing) to increasing water stress. At Moody, SDSU, and Lovjoy,) in grain samples collected at harvest were lower in samples collected from divergent summit/shoulder than convergent footslope areas. Grain samples collected from TE80 did not follow this pattern.

The percentage yield variability explained by using) in a linear equation ranged from 0.01 in TE80 to 80% in Brookings (data not shown). In a combined analysis in the four fields where plant samples were collected at harvest, (Moody, SDSU, Lovjoy, and TE80),) explained 62% of the total yield variability (Fig. 4). This analysis suggests that at Moody, SDSU, and Lovjoy, water stress was a primary factor responsible for reduced soybean yields.

Table 4. The influence of watering soybean plants located in the divergent summit/shoulder and Convergent footslope areas on yield and) at Moody in 2000 and SDSU in 2001.

Landscape position	Treatment	Yield	)
		g plant^{-1}	‰
Moody 2000			
Summit/	No-water	4.73	18.22
shoulder	Water	7.17	18.56
	p level	<0.05	<0.05
Footslope	No-water	6.94	19.69
	Water	7.66	20.01
	p level	ns	ns
SDSU 2001			
Summit/	No-water	5.57	16.55
shoulder	Water	8.70	17.06
	p level	<0.01	<0.01
Footslope	No-water	12.73	19.41
	Water	10.72	19.65
	p level	ns	ns

In legumes, water stress may reduce both N_2 and CO_2 fixation (Serraj and Sinclair, 1996; Serraj et al., 1998). The effect of water stress on photosynthesis is well documented and was discussed earlier in this paper. On the other hand, the effect of water availability on N_2 fixation is less well understood. Serraj et al. (1998) reported that decreases in nodule activity and increases in total non-structural carbohydrates in nodules associated with drought, suggest that plant growth during drought may not be C limited. Purcell and King (1996) reported that due to the extreme sensitivity of nodules to drought, yields can be increased by adding N fertilizer. However, not all soybean cultivars are equally sensitive to water stress. For example, Sall and Sinclair (1991) reported that "Jackson" has a higher tolerance to water stress than other cultivars.

Nitrogen fixation may also limit soybean growth in plants grown in area with moderate high levels of available water. Under these conditions, adding N fertilizer may also increase yield (Wesley et al., 1998). Protein concentrations are likely to be less when the N supply is less than optimum. The relationship between protein,), and water availability in soybean are beyond the scope of this paper and are discussed in a companion paper at this conference (Clay et al., 2002). Nitrogen stress also has the potential to influence). If N stress reduces the production of chlorophyll and other cellular components required for photosynthesis, then in a C_3 plant N stress will increase). However, N stress may only reduces protein and have a minimal impact on yield.

At Moody and SDSU watered soybean plants located in summit/shoulder areas had higher yields and) than non-watered plants. In footslope areas, watering plants did not influence yield or) (Table 4). These findings are consistent with the hypothesis that water stress, either to the plant or to the nodules, reduced yields in the divergent summit/shoulder area.

Similar relationships between) and yields have been observed for other plants. For example, Clay et al. (2001b) reported that 84% of the yield variability of wheat grown under non-N limiting conditions, was explained by the equation: yield (kg ha^{-1}) = =11000+ 884). Based on this equation, water stress reduced), 1.1312‰ for every Mg ha^{-1} loss in wheat yield. Results from this study were similar and show that for every 1 Mg ha^{-1} loss in soybean yield due to water stress,) decreased 2.6‰. The) values where yield loss occurred were different for wheat and soybean. In wheat, a 40% yield loss due to water stress occurred at a) value of 15.8‰ (relative to a yield of 4,900 kg ha with a) value of 18 ‰), whereas in soybean a 40% yield loss occurred at a) value of 17.3‰ (relative to 2730 kg ha at a) value of 20‰). Differences in the relationship between) and yield in the two crops should not be used as evidence that soybean is more or less drought tolerant than wheat.

Crop breeders have had mixed results in using) as a water stress assessment tool (Hall et al., 1994). Mixed results can result from different plant attributes designed to increase water use efficiency having opposite effects on). For example, water use efficiency can be improved by increasing the root depth or early stomatal closure (Ludlow and Muchow, 1990; Muchow and Sinclair, 1991; Sinclair and Muchow, 2001). Increasing the rooting depth should increase available water and Δ in C_3 plants (equation 3), while early stomatal closure should reduce Δ (Equation 3). Clearly, the potential influence of the individual attributes on Δ, yield, and drought tolerance must be understood in order to use) as a diagnostic tool.

In summary, in four fields GDVI, NDVI, and NIR reflectance measured in August were positively correlated to yield. Crop reflectance in the green and red bands were either negatively or not correlated to yield. These results suggest that crop reflectance can be used to assess soybean yield variability. Water stress was responsible for yield reductions in the divergent summit/shoulder area. In the convergent foot-slope position, adding water did not impact yield or). In a combined analysis in the four fields where grain samples were collected at harvest (Moody,SDSU, Lovjoy, and TE80),) explained 62% of the total yield variability. This analysis suggests that at Moody, SDSU, and Lovjoy, water stress reduced summit/shoulder area soybean yields between 25 and 60%, and at TE80, water stress had a limited impact on yield.

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