The Effect of Irrigation and Genotype on Carbon and Nitrogen Isotope Composition in Peanut (*Arachis hypogaea* L.) Leaf Tissue

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ABSTRACT

Water scarcity is a significant problem faced by producers worldwide and is becoming an increasing problem to growers in the U.S. peanut (*Arachis hypogaea* L.) producing areas due to years of drought and increasing urban demands on water resources. Because of this, high water-use efficiency (WUE) has now become a priority in many peanut breeding programs. To support this effort, the variation in WUE, as measured by carbon isotope composition ($\delta^{13}C$), of three commonly grown peanut cultivars was evaluated under differing irrigation environments during 2001 and 2002 at a research farm in Shellman, GA, U.S.A. The specific experimental objectives were: 1) to determine if genetic variability existed in $\delta^{13}C$, $\delta^{15}N$ SLA, and SPAD among three commonly grown U.S. peanut genotypes; 2) to determine if differing irrigation levels affected the pattern of variability; and 3) to quantify the relationship between $\delta^{13}C$ and the measured leaf phenotypic characteristics. During both 2001 and 2002, cv. Georgia Green had significantly higher yields and lower $\delta^{13}C$ and SPAD chlorophyll content than the other two genotypes, and lower %N than the cultivar C99R. Specific leaf area and %C for Georgia Green were significantly greater than for the cultivar AT201. In addition, irrigation treatment significantly affected yield such that the NI (nonirrigated) treatment yields were significantly lower than any of the irrigated treatments (33%, 66%, or 100%). However, the irrigation effects on leaf phenotypic characteristics were less apparent with differences existing only for $\delta^{15}N$, SPAD chlorophyll, and SLA. The correlation of $\delta^{13}C$ and yield was significant for C99R in 2001 and AT201 in 2002, while correlations with $\delta^{13}C$ and the other leaf phenotypic characteristics were scarce. This makes the utility of the traits as easily measured surrogates for WUE very limited.

Key Words: irrigation, water use efficiency, isotope, drought.

Water scarcity is a production problem faced by most peanut growers worldwide. Peanut (*Arachis hypogaea* L.) originates from South America (Hammons, 1982) and is adapted to warm temperatures and long growing seasons. Therefore, peanut is grown almost exclusively in regions prone to extended, or at least partial, periods of drought during the growing season. Irrigation has proven to be a boon to producers, increasing yields by as much as 19% (Lamb et al., 1997), but is often either severely limited or non-existent for many growers. Therefore, research in peanut production needs to be conducted concerning: 1) the sustainable use of limited irrigation; and 2) the development of genotypes that are best adapted to these conditions, particularly those peanut genotypes that are highly water use efficient. However, increased water use efficiency must accompany the maintenance of high yield to have any utility in agricultural systems. The intrinsic physiology of peanut matches this criteria; peanut has the potential to have very high photosynthetic capacity accompanied by low stomatal conductance levels, translating into high water use efficiency without sacrificing carbon assimilation and possibly yield (Wright et al., 1993).

Physiological water use efficiency (WUE) is defined as the ratio of photosynthesis to transpiration and is often a limitation to peanut productivity under drought (Nageswara Rao and Wright, 1994). However, determining the variability in WUE among peanut genotypes or pinpointing the production conditions that significantly affect WUE can be extremely problematic due to the complexity and tedious nature of direct measures of WUE (Wright et al., 1993). Traditional procedures of measuring WUE require weighing lysimeters and accurate measurements of water applied throughout the growing season (Wright et al., 1988; Hatfield et al., 1989). In the last two decades, extensive research has identified an accurate surrogate for WUE in peanut through the measurement of natural $\delta^{13}C$ composition in plant tissue. The isotopic discrimination of ^{13}C that occurs during the photosynthetic pathway in C_3 plants (Farquhar et al., 1982; Farquhar and Richards, 1984) is well correlated with WUE in peanut and provides a long-term measurement of WUE across the season (Wright et al., 1993; Nageswara Rao and Wright, 1994). Water-use efficiency is positively correlated with $\delta^{13}C$

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composition (i.e. the higher the $\delta^{13}C$ composition, the higher the WUE).

While extensive information documenting varietal differences in $\delta^{13}C$ content (and thus WUE) have been conducted in Australia, almost nothing is known about the variability in isotopic composition among genotypes utilized in U.S. peanut producing regions. Surveys of the genetic variation in $\delta^{13}C$ are needed for U.S. peanut genotypes, and the role that environment and production practices, particularly irrigation, play in changing the expression of these genetic differences needs to be quantified. This type of information is invaluable for developing more water use efficient varieties adapted to the U.S. production environment.

Additional information is needed about the interaction of $\delta^{13}C$ with other leaf phenotypic characteristics, particularly $\delta^{15}N$. Theoretical analyses and field data suggest that WUE may interact with nitrogen fixation and other nitrogen nutrition effects (Schulze et al., 1991; Guehl et al., 1998). The overriding pattern appears to be a positive correlation between $\delta^{13}C$ and $\delta^{15}N$, such that N_2 fixation (low $\delta^{15}N$ values) is associated with reduced water-use efficiency (more negative $\delta^{13}C$) (Schulze et al., 1991; Handley et al., 1994; Knight et al., 1993). Therefore, in studies examining $\delta^{13}C$ in a leguminous crop, it is important to likewise examine the relationship between $\delta^{13}C$ and $\delta^{15}N$ and the implications for nitrogen fixation. Lastly, the relationship of $\delta^{13}C$ with the leaf characteristics SPAD chlorophyll content and specific leaf area (SLA) would be important to evaluate because the characteristics are being used in other breeding programs and could potentially be screening tools for WUE (Nageswara Rao and Wright, 1994; Nageswara Rao et al., 1995). None of this information is available for U.S. peanut cultivars or developing breeding lines.

This experiment was conducted in order to document genetic variation and the effect of irrigation environment on $\delta^{13}C$, $\delta^{15}N$, and other leaf characteristics in U.S. grown peanut genotypes and to quantify the relationship between $\delta^{13}C$, SLA, SPAD, and $\delta^{15}N$. The specific experimental objectives were: 1) to determine if genetic variability existed in $\delta^{13}C$, and $\delta^{15}N$ SLA, SPAD among three commonly grown U.S. peanut genotypes; 2) to determine if differing irrigation levels affected the pattern of variability; and 3) to quantify the relationship between $\delta^{13}C$ and several leaf phenotypic characteristics.

Materials and Methods

Field Site. The experiment was conducted during 2001 and 2002 at the USDA-ARS Multicrop Research Farm in Shellman, Georgia, U.S.A. The soil was a Greenville fine sandy loam (fine, kaolinitic, thermic Rhodic Kandiudults) with 0-2% slope. Conventional tillage practices were followed and included: disking, subsoiling, moldboard plowing, field cultivating, rototilling, and planting. Two side by side experimental fields were established at this research farm, one ca. 146 m $\times$ 329 m with overhead (OH) irrigation treatments, and one ca. 49 m $\times$ 329 m as the non-irrigated (NI) treatment. Each field was divided into 16.5 m $\times$ 36.6 m plots containing 18 cropping rows and 2 border rows each containing randomized crop rotations and replications. The field was established in 2001 and followed a fallow history; in 2002, the three peanut replications sampled were following planted cotton in 2001.

The lateral overhead irrigation system contained three equal length spans with approximately 13 nozzles each. To apply differential levels of water to each treatment plot, different sized sprinkler heads were placed on each drop nozzle within a span, such that one span applied a full rate of desired irrigation (100%), the second span applied 66% of this amount, and the third span applied 33% of this amount. Overhead sprinkler irrigation amount and timing were determined by the Irrigator Pro expert system (Lamb et al., 1993; Davidson et al., 1998) based on soil temperature measurements collected in the 100% treatment. Time between irrigation events was typically three to four days during the most active crop growth period of the season. The NI (nonirrigated) block received only rainfall precipitation.

Plant Collection. During the 2001 and 2002 growing seasons, plant samples from three peanut genotypes (cvs. Georgia Green, C99R, and AT201) were taken in four water treatments (100%, 66%, 33%, NI) and three replications. The following traits were measured in both years: $\delta^{13}C$, $\delta^{15}N$, percent carbon (%C), percent nitrogen (%N), SLA, and SPAD chlorophyll content. Leaf samples were collected from six plants spaced along two rows per replication. Sampling was completed in a single day and within the morning hours (800 – 1200). In both years, peanut leaf tissue was collected approximately 90 days after planting. This phenological period is associated with the highest ribulose bisphosphate carboxylase (rubisco) levels and concomitantly the highest photosynthetic levels of the season. Sampling during this time period ensures varietal differences in photosynthesis (and therefore WUE) would be most evident (Nageswara Rao and Wright, 1994; Nageswara Rao et al., 1995). Tissue collection was standardized to second nodal apex leaves that had relatively no insect or

disease damage. Standardizing to the second nodal leaf position has been shown to maximize the relationship between chlorophyll content and specific leaf area (Nageswara Rao et al., 2001). Tetrafoliate leaves were excised and chlorophyll content was measured using the Minolta SPAD (Soil-Plant Analyses Development Unit, Minolta Corp., Ramsey, N.J., U.S.A.) chlorophyll meter directly after removal from the plant. The SPAD chlorophyll meter measures absorbance by plant tissues of wavelengths in the visible spectrum and serves as a measure of the relative internal concentration of chlorophylls a and b. One SPAD chlorophyll reading was taken on each of the four leaflets, avoiding the midrib, and then averaged for one chlorophyll reading per plant to correct for possible non-homogeneous distribution of chlorophyll throughout the leaf (Monje and Bugbee, 1992). Tetrafoliate leaves were then placed on ice and refrigerated at 4 C until further analysis.

Leaves were taken back to the laboratory and hydrated in distilled water for at least three hours prior to leaf area measurement in order to bring them all to a standardized turgor level (Nageswara Rao et al., 2001). Leaflets were removed from each petiole and the leaf area of the four leaflets was measured with an LI-3000A leaf area meter (LI-COR Inc., Lincoln, NE, U.S.A.) and summed to give total leaf area. Leaves were then oven dried at 60 C for 72 hours and weighed. Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry weight. Leaves were then fine ground using a Braun ® (model KSM2) coffee grinder and analyzed for carbon isotope composition ($\delta^{13}C$), $\delta^{15}N$, %C, and %N.

Corrections for vapor pressure deficit (VPD) on SLA were applied following Nageswara Rao et al. (2001); but by replacing the measurement of "prevailing VPD" by logged VPD and incident radiation (R) measured on 15-minute intervals using HOBO ® (Onset Computer Corporation, Bourne, MA, U.S.A.) dataloggers throughout the day prior to tissue sampling. VPD and R values were averaged between the hours of 900 and 1600 (those hours that correspond with the majority of photosynthetic activity of the peanut plant), and the SLA correction from Nageswara Rao et al. (2001) was applied as follows:

$$\text{Corrected SLA} = (\text{SLA} * \text{VPD})/\text{R}$$

This formula is corrected from the original published value (Nageswara Rao, personal communication).

In order to determine the isotopic composition in the peanut samples, leaf tissue was analyzed at the University of Arkansas Stable Isotope Laboratory in 2001 and at the Colorado Plateau Stable Isotope Laboratory, Department of Biological Sciences, Northern Arizona University in 2002. Samples of the ground leaves (2 mg, +/- 0.2 mg) were weighed, sealed in capsules and, along with standards, loaded into the elemental analyzer autosampler (a "Zero Blank" autosampler from Costech Analytical Technologies in Valencia, CA). Samples and standards were combusted in the elemental analyzer (Carlo Erba NC2500 elemental analyzer coupled with a Thermoquest Finnigan Delta plus isotope ratio mass spectrometer). Lab standards, which were calibrated against internationally distributed isotope standards, were analyzed at regular intervals throughout the sample runs. The resulting N_2 and CO_2 gases (along with isotopic reference gases for N_2 and CO_2) were admitted to the mass spectrometer via Finnigan's Conflo II interface. Data were collected and processed by Finnigan's Isodat software. Sample results are based on one analysis per sample ($\delta^{13}C$, $\delta^{15}N$,%N and %C were all determined with the same analysis). Isotope results are reported in delta notation vs. Air (for nitrogen) and vs. PDB (for carbon) in permil. Stable carbon isotope composition was expressed as $\delta^{13}C$ where $\delta^{13}C$ (‰) = [(R sample/R standard)-1] $\times$ 1000, and where R is the $^{13}C/^{12}C$ ratio. Composition of $^{13}C/^{12}C$ ($\delta^{13}C$) rather than discrimination of ^{13}C (Δ) is reported due to the possible differences in atmospheric components linked to natural or man-made C emissions between seasons.

Harvest and Yield Determination. Peanuts were dug using a two-row peanut inverter (Kelly Manufacturing Co., Inc., Tifton, GA, USA). They were allowed to dry in the windrow for approximately 3 days and then harvested with a two row peanut combine (Amadus Industries, Inc., Suffolk, VA). Samples were returned to the laboratory and dried using air and/or heat to less than 10.5% kernel moisture content and weighed. Net pod weight was calculated by deducting the weight of foreign material and excess moisture beyond 7%.

Statistical Analyses. Statistical analyses were performed using JMP SAS (SAS 1997) and SAS (Version 8). Factorial analysis of variance (ANOVA) was used to determine the effect of year, genotype, irrigation, and all possible interactions; the factors of replication and plant nested within replication were also included in the model. Differences among multiple levels of a given factor were determined using a Tukey's HSD multiple comparisons test. Pearson product-moment correlations were used to determine the relationship between $\delta^{13}C$, yield, and leaf phenotypic characteristics.

Table 1. Total water received on field treatment plots (100%, 66%, 33%, and non-irrigated (NI)) during the 2001 and 2002 growing seasons. Total includes water applied through irrigation and received through rainfall; values are in mm ha^{-1}.

Water Treatment	2001	2001
100%	711	627
66%	649	563
33%	588	501
NI	528	439

A drought susceptibility index (S) was calculated for each measured trait following Fischer and Maurer (1978) as presented in Peleg et al. (2005) and expressed as:

$$S = (1 - Y_{dry}/Y_{wet})/(1 - X_{dry}/X_{wet})$$

where Y_{dry} and Y_{wet} are the average values of a given peanut genotype under dryland and irrigated (averaged across water levels) treatments, respectively, and X_{dry} and X_{wet} are the mean values of all genotypes under these treatments, respectively.

Results

Total water received within each water treatment ranged from 711 and 627 mm ha^{-1} in 2001 and 2002, respectively, for the 100% irrigation treatment and 528 and 43 mm ha^{-1} in 2001 and 2002, respectively, for the non-irrigated treatment (Table 1). Water treatment affected peanut yield, with no significant differences between 2001 and 2002. Across years, there were significant differences in yield for peanut genotype (df = 2, F Ratio = 5.9, P value = 0.0048), with Georgia Green having significantly higher yields than the other two genotypes. In addition, irrigation treatment significantly affected yield across genotypes (df = 3, F Ratio = 18.4, P value = 0.0001) such that the NI treatment yields were significantly lower than any of the irrigated treatments (33%, 66%, or 100%; Table 2). In both years the yield in the 100% treatment was numerically lower than the 66% treatment, indicating possible over-watering in the former treatment.

Table 2. Mean yield for three peanut genotypes, Georgia Green, C99R, and AT201, under three irrigation levels, 100%, 66%, 33%, and non-irrigated (NI) during 2001 and 2002 growing seasons. Yield was not measured for C99R in 2002.

	Georgia Green	C99R	AT201
Water Treatment	kg/ha	kg/ha	kg/ha
2001			
100%	5257	4826	4460
66%	5439	5018	4781
33%	4973	4552	4546
NI	3547	3246	3542
2002			
100%	5246	[a]	4372
66%	5367	[a]	4715
33%	5033	[a]	4407
NI	4048	[a]	3373

[a]yield for the genotype C99R not measured in 2002.

Significant differences in $\delta^{13}C$, $\delta^{15}N$, SPAD chlorophyll content, SLA, %C, %N, and C to N ratio were found between years, and differences existed in all traits except $\delta^{15}N$ among genotypes (Table 3). Irrigation effects were less apparent with differences existing only for $\delta^{15}N$, SPAD chlorophyll, and SLA. Year by genotype interactions were significant for both $\delta^{13}C$ and $\delta^{15}N$, while year by irrigation interactions were significant for $\delta^{15}N$, SPAD, specific leaf area, %N, and C to N ratio. There was no significant genotype by irrigation effect. The three-way interaction among year, genotype, and irrigation was significant only for %C (Table 3).

Mean $\delta^{13}C$ was significantly lower (more negative) in 2002 than in 2001; while mean $\delta^{15}N$, specific leaf area, and %N were greater in 2002 (Table 4). Multiple comparisons tests revealed that the significant differences among genotypes for all traits except $\delta^{15}N$ were due to the cultivar Georgia Green having significantly lower $\delta^{13}C$ and SPAD chlorophyll content than the other two genotypes and lower %N than the cultivar C99R; while SLA and %C for Georgia Green were significantly greater than for the cultivar AT201. The significant effects of irrigation treatment revealed higher values of $\delta^{15}N$ and SLA in the NI treatment as compared to the other three irrigation levels, and higher SPAD chlorophyll content in the NI treatment in comparison to the 100% irrigation level. For mean SLA values, the 66% irrigated treatment had the lowest SLA in comparison to the other three treatments (Table 4).

The relationship between $\delta^{13}C$ and peanut yield (across irrigation treatments) is shown in Figure 1 for 2001 and 2002. In 2001, the correlation between $\delta^{13}C$ and peanut yield was only significant for the cultivar C99R, which showed a negative correlation with yield as $\delta^{13}C$ increased (became less negative). In 2002, yield data on C99R was unavailable; however, the correlation between $\delta^{13}C$ and yield was significant for the cultivar AT201, but in this case, was a positive relationship such that $\delta^{13}C$ increased (became less negative) with increasing yield. There were limited significant

Table 3. ANOVA results for the effects of year, genotype, irrigation, and their interactions on leaf physiological traits in peanut. Results reported as F Ratio with associated significance level.

	Traits [a]						
Source of variation	$\delta^{13}C$	$\delta^{15}N$	SPAD	SLA	Perc C	Perc N	C/N
Year	4.5 *	12.1 **	10.1 **	22.8 **	1728.3 **	8.3 **	52.4 **
Genotype	36.6 **	2.3 NS	23.2 **	16.8 **	27.7 **	39.6 **	24.5 **
Irrigation	2.3 NS	27.9 **	3.2 *	111.1 **	0.2 NS	1.3 NS	0.6 NS
Y X Gen	3.9 *	13.5 **	1.6 NS	3.0 NS	0.0 NS	2.0 NS	2.3 NS
Y X Irr	1.3 NS	3.5 *	3.4 *	43.3 **	0.8 NS	4.6 **	4.3 **
Gen X Irr	1.8 NS	1.2 NS	0.2 NS	0.5 NS	1.2 NS	0.8 NS	0.9 NS
Y X Gen X Irr	0.2 NS	1.1 NS	1.8 NS	0.4 NS	2.9 **	0.6 NS	0.7 NS
Rep	2.2 NS	0.8 NS	8.0 **	10.8 **	7.6 **	39.9 **	28.6 **
Plant (Rep)	1.7 *	0.7 NS	1.0 NS	0.4 NS	0.8 NS	1.4 NS	1.1 NS

*Significant at $P < 0.05$; NS, non-significant

**Significant at $P < 0.01$

[a] $\delta^{13}C$, $\delta^{15}N$, carbon and nitrogen isotopic composition of peanut leaf tissue; SPAD, chlorophyll content as measured by the SPAD chlorophyll meter; SLA, specific leaf area corrected by VPD level on the day prior to leaf collection; Perc C, Perc N, percent carbon and nitrogen of leaf tissue; C/N, carbon nitrogen ration of leaf tissue.

correlations with yield for other phenotypic traits (Table 5). For the cultivar AT201, SPAD chlorophyll content in 2001 and 2002 was significantly negatively correlated. None of the other cultivars showed such a relationship. Both C99R and Georgia Green had significant negative correlations of yield with SLA in 2001 and 2002 (with the exception of no measured yield for C99R in 2002). C99R further showed a negative relationship with yield for $\delta^{15}N$; while Georgia Green had a negative and positive relationship with yield for %N and C/N, respectively.

The phenotypic correlations between $\delta^{13}C$ and the other measured traits were equally sparse (Table 5). In 2001, significant positive relationships between $\delta^{13}C$ and SLA for C99R and SPAD chlorophyll for Georgia Green were noted. For Georgia Green in this same year, %C was negatively associated with $\delta^{13}C$. In 2002, SPAD was negatively correlated with $\delta^{13}C$ for AT201, while Georgia Green showed a positive correlation between $\delta^{13}C$ and $\delta^{15}N$. There were no significant correlations between $\delta^{13}C$ and the other phenotypic traits for the cultivar C99R in this same year.

The drought susceptibility index (S) showed interesting differences among cultivars for the various phenotypic traits in both 2001 and 2002 (Figure 2). Overall and with few exceptions, the cultivar AT201 exhibited an advantage (lower values) in the drought susceptibility index in comparison to the other genotypes for $\delta^{13}C$, $\delta^{15}N$, SLA, and SPAD for both years. On the other hand, the cultivar Georgia Green (with the exception of its S value for $\delta^{15}N$ in 2001) exhibited a disadvantage in drought tolerance with its consistently higher S values for the four traits measured. The cultivar C99R primarily exhibited intermediate S values between the cultivars AT201 and Georgia Green for the four traits.

Discussion

Significant variation in isotopic composition and related leaf phenotypic characteristics was found for three *Arachis* genotypes that are currently utilized in the southeastern U.S. production regions. This finding supports the possibility of selecting for U.S. genotypes with increased $\delta^{13}C$ (and thus WUE) in programs aimed at increasing the efficiency of peanut production under water scarce environments. These results also concur with previous studies that found genotypic variation in $\delta^{13}C$ for peanut in greenhouse (Hubick et al., 1986) and field conditions (Nageswara Rao et al., 1993). In peanut, variation in $\delta^{13}C$ appears to be due to variability among genotypes in their photosynthetic capacity and not stomatal factors (Nageswara Rao et al., 1995; Nageswara Rao et al., 2001). The $\delta^{13}C$ content of leaf tissue can be controlled either through an intrinsic photosynthetic capacity (capacitance types) or through stomatal diffusive properties (conductance types) (Udayakumar et al., 1998). In conductance type plants, high $\delta^{13}C$ concentrations, and thus high WUE, is at the expense of dry matter production and yield. In contrast, peanut is a capacitance type plant that has the ability to concentrate $\delta^{13}C$ in its tissues without stomatal closure, thereby making it possible to select for high $\delta^{13}C$ and high WUE while maintaining high growth rates and yield (Udayakumar et al., 1998). Mean differences among genotypes in this study revealed that the cv.

Table 4. Mean [a] values and standard error (in parentheses) for $\delta^{13}C$, $\delta^{15}N$, SPAD, SLA, %C, %N, and C/N ratio. Values presented for each irrigation treatment (100%, 66%, 33%, and non-irrigated (NI)) in both 2001 and 2002 for three peanut genotypes, AT201, Georgia Green, and C99R.

	Trait[b]	Genotype	100%	66%	33%	Dryland
2001						
	$\delta^{15}N$	AT201	0.18 (0.1)	0.60 (0.2)	0.72 (0.1)	1.14 (0.1)
		Georgia Green	0.77 (0.2)	0.74 (0.1)	1.27 (0.2)	1.24 (0.2)
		C-99R	0.19 (0.1)	0.33 (0.1)	0.34 (0.1)	1.02 (0.1)
	SPAD	AT201	42.7 (0.8)	41.4 (0.8)	43.4 (0.4)	44.6 (0.6)
		Georgia Green	40.6 (0.5)	40.3 (0.6)	39.5 (0.7)	42.7 (0.7)
		C-99R	40.9 (0.8)	42.0 (0.7)	42.3 (0.6)	44.3 (0.8)
	SLA	AT201	102.8 (5.1)	83.3 (2.5)	76.9 (2.4)	126.8 (2.9)
		Georgia Green	111.1 (3.7)	96.1 (3.0)	89.0 (3.2)	145.3 (4.6)
		C-99R	116.5 (4.2)	101.6 (3.7)	87.6 (2.9)	146.0 (3.6)
	$\delta^{13}C$	AT201	−26.1 (0.2)	−26.3 (0.2)	−26.3 (0.2)	−26.3 (0.1)
		Georgia Green	−27.0 (0.1)	−27.2 (0.1)	−27.3 (0.1)	−26.8 (0.1)
		C-99R	−26.5 (0.1)	−26.7 (0.1)	−26.6 (0.1)	−26.3 (0.1)
2002						
	$\delta^{15}N$	AT201	0.71 (0.1)	0.82 (0.2)	0.55 (0.1)	1.23 (0.2)
		Georgia Green	0.54 (0.1)	0.50 (0.1)	0.65 (0.1)	1.70 (0.3)
		C-99R	1.11 (0.2)	0.89 (0.1)	0.83 (0.2)	1.90 (0.4)
	SPAD	AT201	41.3 (0.8)	43.3 (0.8)	42.1 (1.0)	43.5 (1.4)
		Georgia Green	37.3 (0.7)	39.0 (1.4)	40.4 (1.3)	38.0 (1.2)
		C-99R	41.6 (1.0)	42.0 (0.9)	40.8 (1.2)	41.0 (1.4)
	SLA	AT201	92.5 (5.6)	96.2 (4.0)	125.3 (8.8)	131.7 (5.0)
		Georgia Green	96.6 (3.4)	96.5 (2.3)	121.1 (4.7)	140.6 (3.4)
		C-99R	106.6 (5.1)	102.8 (3.7)	133.7 (7.3)	141.4 (4.7)
	$\delta^{13}C$	AT201	−26.5 (0.2)	−26.6 (0.2)	−26.6 (0.2)	−26.8 (0.2)
		Georgia Green	−27.1 (0.1)	−27.0 (0.1)	−27.3 (0.1)	−26.8 (0.2)
		C-99R	−26.7 (0.2)	−26.4 (0.2)	−26.8 (0.2)	−26.5 (0.1)

[a]Within each year, means without a common letter are significantly ($P < 0.05$) different according to Tukey's multiple comparisons test.

[b]$\delta^{13}C$, $\delta^{15}N$, carbon and nitrogen isotopic composition of peanut leaf tissue; SPAD, chlorophyll content as measured by the SPAD chlorophyll meter; SLA, specific leaf area corrected by VPD level on the day prior to leaf collection; Perc C, Perc N, percent carbon and nitrogen of leaf tissue; C/N, carbon nitrogen ration of leaf tissue.

Georgia Green had significantly lower $\delta^{13}C$, and thus WUE, in comparison to the other two genotypes.

While the current results agree with findings of variability in $\delta^{13}C$ among peanut genotypes, they counter many studies examining irrigation effects on $\delta^{13}C$ in other crop species. What was surprising in this study was the lack of effect of irrigation on the expression of $\delta^{13}C$, which is contrary to other studies in crops such as wheat, cotton, (Yakir et al., 1990; Saranga et al., 1998; Monneveux et al., 2005; Merah et al., 1999) and even loblolly pine (Choi et al., 2005). In these studies, $\delta^{13}C$ was increased under non-irrigated or drought stressed conditions, indicating that WUE increases under water scarcity. In the current study, the only significant environmental effects on $\delta^{13}C$ were reflected through the significant effect of year, indicating the climatic variation between 2001 and 2002 had an effect on $\delta^{13}C$. Further, the existence of a significant G X E interaction between genotype and year for $\delta^{13}C$ (Table 3) indicated that the genetic effects on $\delta^{13}C$ were strong, but environment played a role as well. Evidence for G X E interactions for WUE in peanut is scarce (Hubick, 1990; Nageswara Rao and Wright, 1994). A few previous studies in peanut documented that variation in $\delta^{13}C$ was influenced by location and genotype (Nageswara Rao and Wright, 1994; Brown and Byrd, 1996); while Wright et al. (1988) found strong genetic control over $\delta^{13}C$ with little effect of the environment in four peanut genotypes when grown either in open or closed canopies.

There were significant genetic and environmental effects on the other phenotypic traits measured. Genetic variation was strong for all the leaf characteristics except $\delta^{15}N$; in addition, significant variation among years was present indicating an environmental component to control of the expression of these traits. But in this instance, irrigation environment also had a significant role in de-

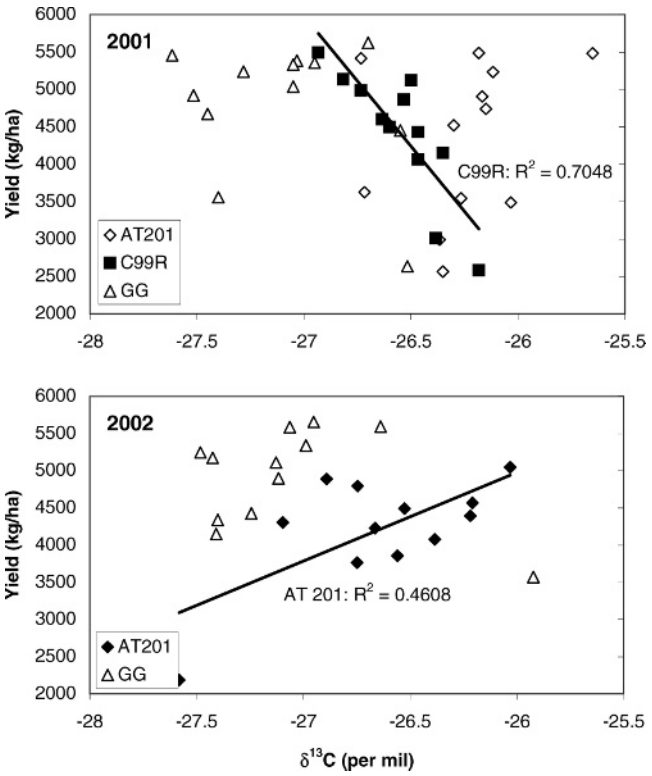

Fig. 1. Relationship between peanut yield and leaf carbon isotope content in 2001 and 2002 for three cultivars: AT201, C99R, and Georgia Green (GG).

termining the variability in $\delta^{15}N$, SPAD, and SLA. Irrigation, in general, tended to decrease $\delta^{15}N$, SPAD, and SLA. For $\delta^{15}N$, these results are in line with the study of Handley et al. (1999) that found the natural abundance of $\delta^{15}N$ in leaf tissue decreased with increasing soil moisture and therefore, was reflective of water availability at a given site. Our results for SLA, however, are contrary to most studies. Decreased SLA under limited irrigation is a common finding in many drought studies (Marcelis et al., 1998), and may be a mechanism of increasing water use efficiency through the concentration of chlorophyll and proteins in thick leaves leading to greater photosynthetic capacity (Liu and Stutzel, 2004). The SPAD chlorophyll content results in this study do show that the highest chlorophyll concentrations are in leaves in the NI treatment, but are concentrated in thinner leaves in comparison to the irrigated treatments. Given the low SLA in the irrigated treatments and the lack of irrigation effect on $\delta^{13}C$, severe drought stress was likely not present in either 2001 or 2002.

The relationship between $\delta^{13}C$ and yield was complicated for the genotypes tested and appeared to vary among the cultivars. The cultivar Georgia Green showed no significant correlation with $\delta^{13}C$ and yield in either 2001 or 2002. A reduced correlation between $\delta^{13}C$ and yield in wheat has been linked to an absence of variability in $\delta^{13}C$. This can be explained by increased photosynthetic capacity being offset by increases in stomatal aperture, leading to lower variation in $\delta^{13}C$ and reduced correlation between $\delta^{13}C$ and yield (Monneveux et al., 2005). This may indicate that Georgia Green tends to be a conductance physiological type instead of a capacitance type. The high relative drought susceptibility index values (S) for Georgia Green for the trait $\delta^{13}C$ also indicate that this genotype is more drought susceptible, and indeed its mean $\delta^{13}C$ values signify lower WUE than the other genotypes. The cultivar C99R showed a negative correlation with $\delta^{13}C$ and yield, indicating that this genotype may also be acting as a conductance type, where $\delta^{13}C$ (and thus WUE) is

Table 5. Correlations between leaf $\delta^{15}N$, percent C and N, C to N ratio, specific leaf area, and SPAD chlorophyll content with yield and $\delta^{13}C$ in 2001 and 2002 for three peanut genotypes.

	Genotype		
	AT201	C99R	Georgia Green
Phenotypic correlation with yield			
2001			
$d^{15}N$	−0.26 NS	−0.77 **	−0.27 NS
%C	−0.46 NS	−0.28 NS	−0.10 NS
%N	−0.50 NS	−0.38 NS	−0.80 **
C/N	0.46 NS	0.07 NS	0.77 **
SLA	−0.46 NS	−0.66 *	−0.85 **
SPAD	−0.78 **	−0.26 NS	−0.54 NS
2002			
$d^{15}N$	−0.46 NS	[a]	−0.67 *
%C	−0.60 *	[a]	0.53 NS
%N	−0.35 NS	[a]	−0.19 NS
C/N	0.21 NS	[a]	0.24 NS
SLA	−0.52 NS	[a]	−0.75 **
SPAD	−0.65 *	[a]	−0.15 NS
Phenotypic correlation with $d^{13}C$			
2001			
d15N	−0.09 NS	0.55 NS	−0.48 NS
%C	0.10 NS	0.15 NS	−0.67 *
%N	−0.14 NS	0.25 NS	0.34 NS
C/N	0.21 NS	0.00 NS	−0.36 NS
SLA	0.06 NS	0.69 *	0.39 NS
SPAD	−0.49 NS	0.14 NS	0.75 **
2002			
d15N	−0.13 NS	0.52 NS	0.77**
%C	−0.54 NS	−0.25 NS	0.16 NS
%N	−0.42 NS	0.10 NS	0.07 NS
C/N	0.33 NS	−0.14 NS	−0.02 NS
SLA	−0.51 NS	−0.15 NS	0.00 NS
SPAD	−0.65 *	0.05 NS	−0.05 NS

*, **, significant at $P < 0.05$ and 0.01, respectively; NS, non-significant.

[a]yield for the genotype C99R not measured in 2002.

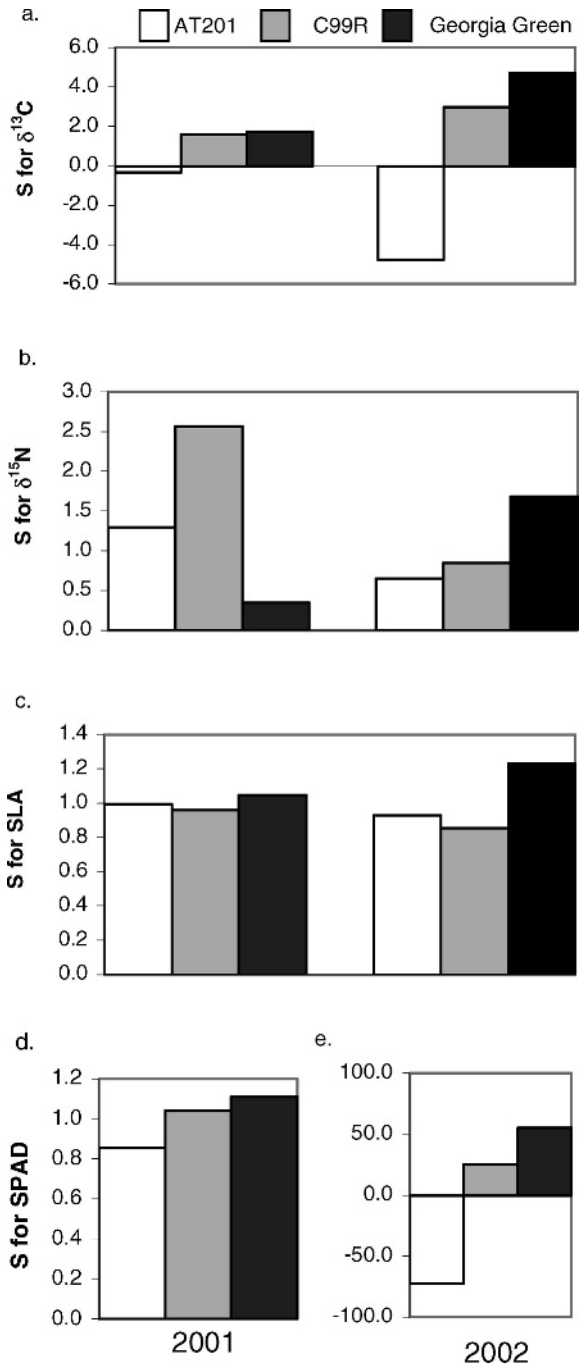

Fig. 2. Susceptibility index (S) for $\delta^{13}C$, $\delta^{15}N$, SLA, and SPAD chlorophyll content for three peanut cultivars: AT201, C99R, and Georgia Green (GG). Low S indicates relative drought tolerance, i.e. trait values in the non-irrigated treatment are similar to values in irrigated treatments.

controlled predominantly through stomatal aperture. In the case of C99R, WUE increases only through stomatal closure and so an increase in WUE brings a concomitant decrease in yield. On the other hand, the genotype AT201 appears to be acting as a capacitance type where increased $\delta^{13}C$ (and thus WUE) is correlated positively with yield in 2004, likely due to an increase in photosynthetic capacity. The low drought tolerance index values for $\delta^{13}C$ also indicate that AT201 may be more water use efficient than the other genotypes, possibly through increased photosynthetic capacity.

The relationships between $\delta^{13}C$ and yield with the other leaf phenotypic characters were extremely limited. Very few traits correlated directly with yield, with the exception of nitrogen related traits ($\delta^{15}N$, and %N) for Georgia Green. Specific leaf area was the most consistent correlation with yield for genotypes and years, indicating that thicker leaves were correlated with higher yields. The relationships with $\delta^{13}C$ and leaf phenotypic characters were nearly nonexistent. In 2002, the relationship between $\delta^{15}N$ with $\delta^{13}C$ was significantly positive for Georgia Green. Very few studies have related $\delta^{15}N$ with $\delta^{13}C$, and those that have, show the relationship to be affected by environment or nonexistent (Guehl et al., 1998). The paucity of significant correlations between $\delta^{13}C$ and SPAD or SLA is unfortunate. In Australia and India, research has confirmed the relationship between SLA and SPAD, two very easily measured characters, with carbon isotope discrimination (Wright et al., 1993; Nageswara Rao and Wright, 1994; Nageswara Rao et al., 1995). This relationship has been purported as a useful, inexpensive, and easily utilized tool to screen large numbers of breeding lines for water use efficiency. However, this study has failed to show a consistent relationship between the two traits in a field setting for these three peanut cultivars. Nageswara Rao et al. (2001) also showed a link between SLA and SPAD chlorophyll content, and it was assumed that because $\delta^{13}C$ and SLA are related, $\delta^{13}C$ and SPAD would be related as well. However, in this study, the correlation between $\delta^{13}C$ and SPAD was significant only for Georgia Green in 2001 and AT201 in 2002, with the direction of the correlation being inconsistent between cultivars. Overall, it appears that the utility of SLA and SPAD chlorophyll for WUE screening tools in U.S. peanut genotypes in a field environment is limited because of the weak correlations between $\delta^{13}C$ and SLA or SPAD.

In conclusion, this study has documented variation in $\delta^{13}C$, $\delta^{15}N$, and other leaf phenotypic characteristics among currently grown U.S. peanut genotypes. Based on the copious number of studies documenting the plasticity of $\delta^{13}C$ in other crop species, it is surprising that there was no significant effect of irrigation on the trait in this study. It might be argued that the crop received adequate water through precipitation regardless of supplemental irrigation level in both years, thus negating the effect of irrigation on $\delta^{13}C$. However, the differences in yields among the irrigation levels in

both 2003 and 2004 somewhat refute this explanation. Perhaps the explanation lies in a relatively strong genetic control for $\delta^{13}C$ in peanut as compared to other crops, making the success of breeding for increased WUE in peanut quite achievable.

Acknowledgments

We thank Kathy Gray who provided invaluable field collection, sample processing, expertise and, in general, made this research possible. We thank Latoya Rucker for sample preparation and Jessie Childre for plot establishment and maintenance.

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