

Peanut Nitrogen Fixation (C_2H_2 Reduction) Response to Soil Dehydration

T.R. Sinclair¹*, A.A. Leilah², and A.K. Schreffler³

ABSTRACT

Peanut (*Arachis hypogaea* L.) is commonly grown on sandy soils that are susceptible to water deficits. Because symbiotic nitrogen fixation in other grain legumes is especially sensitive to soil drying, the sensitivity of peanut nitrogen fixation to water deficits might be an important limitation on peanut production. This greenhouse study was undertaken to observe the response of nitrogen fixation (acetylene reduction) in six peanut cultivars to soil drying. In contrast to other grain legumes, peanut nitrogen fixation was relatively insensitive to soil drying. Acetylene reduction rates did not begin to decrease until soil water deficits had decreased to where leaf gas exchange was affected. These data indicated that restricted nitrogen fixation during drought stress is not likely to be a serious problem in peanut. Nevertheless, cultivar variation in drought sensitivity was identified

indicating that genetic selection might further decrease the susceptibility of peanut nitrogen fixation to drought.

Key Words: Acetylene reduction, nodules, drought, water deficits, transpiration.

Soil dehydration has been shown to adversely affect the symbiotic nitrogen fixation rates in a number of grain legumes both in controlled experiments (Sinclair *et al.*, 1987b; Sall and Sinclair, 1991) and field experiments (Sinclair *et al.*, 1987a). Commonly these experiments have indicated that nitrogen fixation rates decline early in the soil dehydration cycle, preceding all other measures of drought stress. Because peanut is frequently grown on sandy droughty soils, a high sensitivity to soil dehydration in peanut might prove to be an especially restrictive trait.

While nitrogen fixation of well-watered peanut plants has been studied (e.g., Sen and Weaver, 1984), only a limited amount of data has been obtained on the nitrogen fixation activity in drought-stressed peanut. DeVries *et al.* (1989a,b) compared the nitrogen fixation rates and nitrogen accumulation of field-grown peanut with those

¹USDA-ARS, Agronomy Dept., Univ. of Florida, Gainesville, FL 32611-0840.

²Agronomy Dept., Mansoura Univ., El-Mansoura, Egypt.

³Agronomy Dept., Univ. of Florida, Gainesville, FL 32611-0840.

*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply approval or the exclusion of other products that may be suitable also.

¹Corresponding author.

of soybean (*Glycine max* L.) and pigeon pea (*Cajanus cajan* L.). Both types of data indicated decreased nitrogen fixation activity by peanut when the plants were subjected to drought, but the drought sensitivity of peanut tended to be less than that of soybean and pigeon pea. In a series of pot experiments, sensitivity of nitrogen fixation to drought in peanut was also shown (Venkateswarlu *et al.*, 1990; Venkateswarlu *et al.*, 1991). Also, Venkateswarlu *et al.* (1989) concluded that nitrogen fixation in peanut was less sensitive to drought than in cowpea (*Vigna unguiculata* L.).

In the previous studies of peanut nitrogen fixation under drought, the measurements of nitrogen fixation activity were done by incubating detached roots for an hour or more in an atmosphere containing acetylene. It has been shown that disturbance of plants, particularly removal of the roots, can greatly influence nitrogen fixation measurements (Minchin *et al.*, 1986; Sung *et al.*, 1991). Also, long-term exposure of nodules to acetylene can disrupt the initial acetylene reduction rates which actually reflect nodule activity (Minchin *et al.*, 1983). Therefore, the techniques used previously may not have allowed accurate *in situ* measures of nitrogen fixation rates for the stressed peanut plants.

A further complication in the previous studies is the manner in which the drought stress levels in the plants were quantified. Plant stress was determined by measures of leaf water potential, and commonly the leaf water potentials were reported to be at very low levels. While the measure of leaf water potentials may be useful for comparing stress levels among treatments in the same experiment, leaf water potential remains an ambiguous variable for defining absolute stress levels (*e.g.*, Sinclair and Ludlow, 1985). Further, the relationship between leaf water potential and nodule water status is unknown in peanut. Consequently, the previous observations failed to provide an unambiguous measure of drought stress levels which would allow nitrogen fixation responses to be compared among cultivars, species, and drought treatments.

The objective of this study was to characterize the nitrogen fixation activity of several cultivars of peanut in response to differing levels of soil dehydration. Specifically, the experiments were designed to determine if nitrogen fixation rates in peanut are more sensitive to drought stress than transpiration rates, as has been observed in other grain legumes. The nitrogen fixation rates were monitored in a system which allowed intact plants to be repetitively measured through a dehydration cycle. The nitrogen fixation rates were expressed as a function of soil water content because soil water content has been useful as a stable, independent variable for describing the drought response of other physiological traits (*e.g.*, Sinclair *et al.*, 1987b).

Materials and Methods

Six peanut lines were tested for their nitrogen fixation response to soil dehydration; including two cultivars (Florunner and Early Bunch) and four genotypes which had been identified (pers. commun., J.M. Bennett, Univ. of Florida) as drought-susceptible [Pearl Early Runner, VA

83510-2, VA 84090, and VP 8433]. The lines were tested in individual experiments performed in a greenhouse from Sept. 1993 to Feb. 1994. Temperature in the greenhouse was allowed to vary between 20 and 30 C and the plants were exposed to natural irradiance.

The plants were grown in the pot system described by Sall and Sinclair (1991). Briefly, the pots were constructed from 10-cm diameter PVC tubing cut into 30-cm lengths. The bottom of the pots was sealed with an end cap which was fitted with a 3-mm Swagelok elbow fitting (Crawford Fitting Co., Solon, OH) that served as a drainage hole during the growth phase, and as the gas inflow port during nitrogen fixation measurements. The top of the pots was fitted with a toilet flange fitting to which a lid that sealed the pot during acetylene reduction measurements could be attached.

Each pot was filled with approximately 3 kg of sandy loam potting soil. Three seeds were sown in each pot and inoculated with a commercial inoculum (The Nitragin Co., Milwaukee, WI) which resulted in good nodulation for all six lines. Approximately 2 wk after sowing the pots were thinned to a single plant.

Each line was studied in individual experiments, so a group of plants for each line were grown under well-watered conditions for 4 or 5 wk. After this initial growth, nine plants were selected for the drought response test. The lids which sealed the pots for the acetylene assay were attached to the top of the pots and left attached throughout the experiment.

The evening before the acetylene measurements were begun the pots were over watered and allowed to drain overnight. The following morning the pots were weighed to determine the drained soil weight. Six of the nine pots were allowed to dry during the succeeding 2 wk while three pots were maintained as well-watered controls. The pots were weighed each afternoon and the difference between successive daily measurements was used to calculate daily transpiration rate for each pot. The daily increase in plant weight would have caused only a very small underestimate in the daily estimate of transpiration rate. The normalization procedure described below would have further minimized this small bias. Each afternoon after weighing the pots, the well-watered controls were watered to return the pot weight to 250 g less than the drained weight once they had initially lost 250 g of water.

The transpiration rates varied greatly among days mainly as a result of large differences in the natural irradiance. To eliminate this variation, the transpiration rates of the plants subjected to soil drying were normalized against the transpiration rates of the well-watered controls. The daily water loss of each drying pot was divided by the mean water loss of the three well-watered controls.

The acetylene reduction (AR) rates were measured each midday for each of the nine pots. On the day prior to the beginning of the drought experiments, a lid was secured to the top of each pot. Each day a gas mixture of 1 volume C₂H₂:9 volumes air was flowed through the sealed pot at 1 L min⁻¹. The acetylene:air mixture was flowed through the pots for 10 min to allow equilibration in the ethylene outflow. Following the 10-min equilibration period, three gas samples were collected from the gas exit port in the lids of each pot with 1 cm³ syringes. The collection of gas samples usually took 3 to 5 min. Therefore, this system had the important advantages of allowing AR measures to be made using a flow through system on intact plants which

had been exposed only briefly to acetylene.

Once the gas sampling was completed for all pots, the acetylene was removed from the gas flow and the pots were flushed with only air for at least another 45 min to remove the acetylene from the pots. The gas samples were analyzed for their ethylene content using a gas chromatograph with a flame ionization detector (Model 5710A, Hewlett-Packard Corp., Palo Alto, CA). The mean ethylene content of the three gas samples was calculated for each pot. The daily mean ethylene content for each drying pot was normalized against the average ethylene content from the three well-watered controls.

Transpiration and AR rates for each drought-stressed plant were expressed as a function of soil water content as defined by the fraction of transpirable soil water (Sinclair and Ludlow, 1986). The fraction of transpirable water (FTSW) was calculated for each of the dehydrating pots by first determining the difference in pot weight between the drained weight and the weight when the transpiration rate had decreased to, or less than, 10% of the well-watered pots. The FTSW on each day was calculated from the weight measurements as the fraction of the transpirable soil water that still remained in the soil. Therefore, FTSW equal to 1.0 was field capacity and FTSW equal to 0.5 meant that half the available transpirable soil water remained in the soil. The transpiration rates and acetylene reduction rates were plotted against the FTSW calculated for each pot on each day.

Results and Discussion

The response of peanut transpiration rates to dehydrating soil were consistent with that observed for other plants, including other grain legumes (Sinclair and Ludlow, 1986). No change in relative transpiration rates were observed until FTSW decreased to less than 0.4, as illustrated in Fig. 1A for Florunner, Expt. 1. There was a fairly steady decline in transpiration rate until FTSW reached zero. No difference was detected among lines in their transpiration response to soil dehydration. Consequently, it does not appear that the difference in drought tolerance ascribed to the lines can be attributed to differences in the transpiration response as soils dehydrate.

Even though the AR data were scattered, no trend in declining AR rates was apparent until FTSW had decreased to very low levels (Fig. 1B). In fact, it appeared that FTSW had to decrease to about 0.15 before any noticeable trend in decreased AR rates was apparent. Even at very low FTSW the relative AR rates were still quite high. In most cases, soil dehydration was not sufficient to cause nitrogen fixation rates to be any lower than 1/3 of the well-watered plants.

The response of peanut nitrogen fixation to soil dehydration is in marked contrast to that observed for other grain legumes. AR rates in soybean (Sall and Sinclair, 1991), and cowpea and mung bean (Sinclair *et al.*, 1987b) decreased at FTSW of about 0.5. Therefore, in these other species the decline in AR activity preceded the decline in transpiration rates. In contrast, there was no decline in peanut AR rates until values of FTSW were reached that had already resulted in decreased transpiration rates.

To better compare the sensitivity to soil dehydration

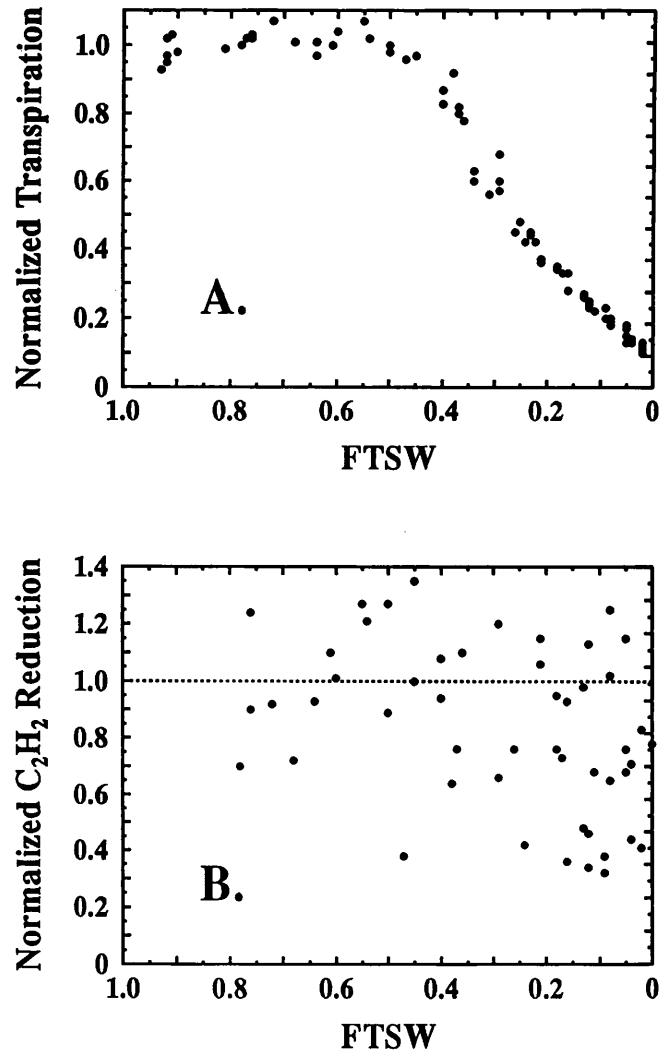


Fig. 1. Individual daily observations (A) normalized transpiration rates and (B) normalized acetylene reduction rates plotted against fraction of transpirable soil water (FTSW) for cultivar Florunner, Expt. #1 during a dehydration cycle. Horizontal dashed line in each panel is at normalized response value of 1.0 indicating no inhibition of activity.

of AR rates relative to transpiration rates, these two variables were plotted against each other directly (Fig. 2). For each day, the normalized AR rate was plotted against the normalized transpiration rate for each plant. If AR and transpiration were equally sensitive to drought, both variables would decrease simultaneously as the soil dried and the data would fall on the 1:1 line. However, if AR was less sensitive to soil dehydration, then the data would fall above the 1:1 line. That is, data above the 1:1 line were obtained when normalized AR rate was maintained at a higher level than normalized transpiration rate.

The plot of normalized AR rate against normalized transpiration rate for Florunner, Expt. 1 clearly illustrated the relative insensitivity of AR to drought conditions (Fig. 2). Virtually, all the data fell above the 1:1 line indicating sustained rates of AR even though transpira-

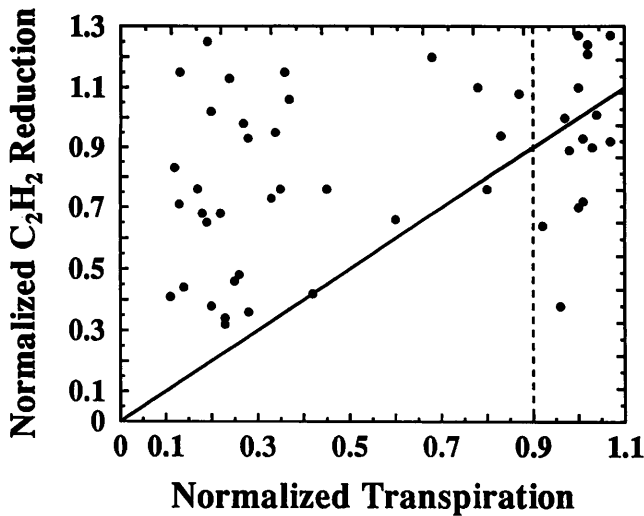


Fig. 2. Normalized acetylene reduction rates plotted against normalized transpiration rates of individual daily observations for cultivar Florunner, Expt. #1 during a dehydration cycle. Vertical dotted line at FTSW equal to 0.9 represents division below which data were tabulated to assess relative sensitivity to water deficits.

tion rates were declining. Substantial decreases in AR rates were not obtained until transpiration rate for the drought-stressed plants was less than about 0.3 of the well-watered plants. These results further illustrate the unusual tolerance of nitrogen fixation in peanut to soil water deficits as compared to other grain legumes.

The comparison between normalized AR rate and normalized transpiration rate was done for each of the six lines. The percentage of data above and below the 1:1 line were calculated as an index of drought response for each line. In this analysis, only data with normalized transpiration rates below 0.9 indicating water-deficit effects in the leaves, were included to avoid biasing the data with a large number of data obtained before drought conditions developed in a pot (Fig. 2). The results of this analysis for each of the six lines are presented in Table 1.

Florunner showed the least sensitivity in normalized AR rates among the six lines tested (Table 1). Normalized AR rates were less than the normalized transpiration rates in very few cases for Florunner. Nitrogen fixation activity, therefore, is predicted not to be a particularly restrictive trait for Florunner peanut when grown under water deficit conditions.

All peanut cultivars tested showed a substantial level of insensitivity of AR to drought stress. Even those cultivars that were originally identified as being drought susceptible, were found to have AR activity that was at least as tolerant to water deficits as was transpiration. Only VP 8433 was found to have an approximately equal sensitivity of AR and transpiration to drought conditions.

The data from these experiments clearly demonstrated that AR in peanut is insensitive to drought stress as compared to transpiration rates. Although this result is in marked contrast to observations reported for other grain legumes, it is not inconsistent with previous studies with peanut. Venkateswarlu *et al.* (1989) found under

Table 1. Comparison of nitrogen fixation and transpiration responses to soil dehydration for six lines of peanut. The percent of observations are presented in which nitrogen fixation is less (> 1:1) or more (< 1:1) sensitive to soil dehydration than transpiration.

Line	no.	N Fixation : Transpir.	
		> 1:1	< 1:1
----- % -----			
Florunner			
Expt. #1	33	97	3
Expt. #2	47	92	8
VA 83510-2	23	83	17
VA 84090	16	81	19
Pearl Early Runner	19	68	32
Early Bunch	23	61	39
VP 8433	15	53	47

greenhouse conditions with peanut that stomatal conductance was much more sensitive to soil dehydration than AR activity. Importantly, DeVries *et al.* (1989a) found for drought-stressed and well-watered, field-grown peanut that statistically significant decreases in AR rates were not obtained until the stomatal conductance of the drought-stressed plots were nearly an order of magnitude less than the well-watered plots.

There are a number of possible explanations for the relative insensitivity of nitrogen fixation to soil drying in peanut as compared to other grain legumes. Because many grain legumes show sensitivity to soil drying well in advance of other physiological processes, the response may well be under active chemical control. One possibility to explain the difference between peanut and other grain legumes may be associated with peanut not transporting nitrogen products from the nodule in the form of ureides (Peoples *et al.*, 1986; Peoples *et al.*, 1991). Experimental evidence in soybean indicated the possibility that ureides may have an important feedback role in regulating the overall metabolism of nodules (Silsbury *et al.*, 1986; Parsons *et al.*, 1993). Assuming that soil drying could result in early inhibition of nitrogen fixation via ureide activity, then peanut would be exempted from this hypothetical feedback and could sustain the observed nitrogen fixation activity at low soil water contents.

Literature Cited

DeVries, J.D., J.M. Bennett, S.L. Albrecht, and K.J. Boote. 1989a. Water relations, nitrogenase activity and root development of three grain legumes in response to soil water deficits. *Field Crops Res.* 21:215-226.

DeVries, J.D., J.M. Bennett, K.J. Boote, S.L. Albrecht, and C.E. Maliro. 1989b. Nitrogen accumulation and partitioning by three grain legumes in response to soil water deficits. *Field Crops Res.* 22:33-44.

Minchin, F.R., J.E. Sheehy, and J.F. Witty. 1986. Further errors in the acetylene reduction assay: Effects of plant disturbance. *J. Exp. Bot.* 37:1581-1591.

- Minchin, F.R., J.F. Witty, J.E. Sheehy, and M. Muller. 1983. A major error in the acetylene reduction assay: Decreases in nodular nitrogenase activity under assay conditions. *J. Exp. Bot.* 34:641-649.
- Parsons, R., A. Stanforth, J.A. Raven, and J.I. Sprent. 1993. Nodule growth and activity may be regulated by a feedback mechanism involving phloem nitrogen. *Plant Cell Environ.* 16:125-136.
- Peoples, M.B., C.A. Atkins, J.S. Pate, K. Chong, A.W. Faizah, P. Suratmini, D.P. Nurhayati, D.J. Bagnall, and F.J. Bergersen. 1991. Re-evaluation of the role of ureides in the xylem transport of nitrogen in *Arachis* species. *Physiol. Plant.* 83:560-567.
- Peoples, M.B., J.S. Pate, C.A. Atkins, and F.J. Bergersen. 1986. Nitrogen nutrition and xylem sap composition of peanut (*Arachis hypogaea* L. cv. Virginia Bunch). *Plant Physiol.* 82:946-951.
- Sall, K., and T.R. Sinclair. 1991. Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration. *Plant Soil* 133:31-37.
- Sen, D., and R.W. Weaver. 1984. A basis for different rates of N_2 -fixation by the same strains of *Rhizobium* in peanut and cowpea nodules. *Plant Sci. Lett.* 34:239-246.
- Silbury, J.H., D.W. Catchpole, and W. Wallace. 1986. Effects of nitrate and ammonium on nitrogenase (C_2H_2 reduction) activity of swards of subterranean clover, *Trifolium subterraneum* L. *Aust. J. Plant Physiol.* 13:257-273.
- Sinclair, T.R., and M.M. Ludlow. 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. *Aust. J. Plant Physiol.* 12:213-217.
- Sinclair, T.R., and M.M. Ludlow. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. *Aust. J. Plant Physiol.* 13:329-341.
- Sinclair, T.R., R.C. Muchow, J.M. Bennett, and L.C. Hammond. 1987a. Relative sensitivity of nitrogen and biomass accumulation to drought in field-grown soybean. *Agron. J.* 79:986-991.
- Sinclair, T.R., R.C. Muchow, M.M. Ludlow, G.J. Leach, R.J. Lawn, and M.A. Foale. 1987b. Field and model analysis of the effect of water deficits on carbon and nitrogen accumulation by soybean, cowpea, and black gram. *Field Crops Res.* 17:121-140.
- Sung, L., A.H. Moloney, S. Hunt, and D.B. Layzell. 1991. The effect of excision on O_2 diffusion and metabolism in soybean nodules. *Physiol. Plant.* 83:67-74.
- Venkateswarlu, B., M. Maheswari, and N. Saharan. 1989. Effects of water deficit on $N_2(C_2H_2)$ fixation in cowpea and groundnut. *Plant Soil.* 114:69-74.
- Venkateswarlu, B., M. Maheswari, and G. Subba Reddy. 1991. Effect of water stress on N_2 -fixation and N-partitioning in groundnut in relation to kernel yield. *Indian J. Exp. Biol.* 29:272-275.
- Venkateswarlu, B., N. Saharan, and M. Maheswari. 1990. Nodulation and $N_2(C_2H_2)$ fixation in cowpea and groundnut during water stress and recovery. *Field Crops Res.* 25:223-232.

Accepted 27 November 1995