The Influence of Genotype on Diurnal and Seasonal Patterns of Nitrogen Fixation in Southernpea (Vigna unguiculata (L.) Walp)¹

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Abstract. Diurnal and seasonal patterns of nitrogen fixation \( \text{N}_2(\text{C}_2\text{H}_2) \) in southernpea were delineated, using 7 genotypes which differed in potential to fix nitrogen. Diurnal activity peaked at 1200 hours, at both 34 and 53 days after planting (DAP). Significant differences in total activity between genotypes were observed, with maximum activity generally coincident with full flowering. High fixing genotypes were higher in total nitrogenase activity throughout the growing season, than were the low fixing genotypes. Peak activity for the latter was found at 34 DAP, while the former peaked at 46 DAP. Mean nodule mass, nodule number and plant dry weight were greatest 53 DAP.

Diurnal and seasonal patterns of \( \text{N}_2 \) fixation (acetylene reduction) have been reported for several legumes including Vigna unguiculata (1), Glycine max (L.) Merrill (1), Pisum sativum L. (6), Arachis hypogea L. (2), Medicago sativa L. (7), Phaseolus vulgaris L. (3, 4) and certain non-legumes (8). As early as 1952, it was shown that such patterns exist and might be influenced by many factors (5). However, few reports have been found regarding the role of plant genotype in these patterns (1, 3, 4). The purpose of this investigation was to determine the influence of southernpea genotypes, with differential \( \text{N}_2 \) fixing potential (9), on diurnal and seasonal patterns of \( \text{N}_2 \) fixation.

Materials and Methods

Diurnal study. Two southernpea cultivars, representing high ('H-Brown Crowder') and low ('L-Bush Purple Hull') \( \text{N}_2 \) fixing potential (9), were hand planted and inoculated with a \( \text{N}_2 \) fixing Micrococcus sp. on diurnal and seasonal patterns of \( \text{N}_2 \) fixation. Phaseolus vulgaris L., was hand planted and inoculated with \( \text{N}_2 \) fixing Rhizobium on a commercial mixed strain Rhizobium inoculant in the field, at College Station, Texas on May 8, 1978. The soil type was a Lufkin fine sandy loam and the experimental design was a randomized block with 5 replications. Seeds were planted in rows 6 m long and 102 cm apart, and thinned to a within row spacing of 15 cm 7 days after emergence. A banded preplant application of ON–19.8P–OK superphosphate fertilizer was applied at the rate of 45 kg/ha. A preplant application of treflan was applied at 1 liter/ha and irrigation water was used as needed. Beginning at 0800 on June 12 and every 4 hr until 0400 on June 13, 5 plants of each genotype were harvested from each block. Soil and air temperature were also determined at each harvest. Plants were severed at the soil line and the tops dried and weighed. The intact root nodule complex was then carefully dug from the field and individually placed into 0.48 liter septum fitted, gas tight canning jars, which were then injected with 25 cc of acetylene (5%). These roots were then incubated for 1 hr at 25°C. A 15 cc sample was removed by syringe and injected into a 10 cc vacutainer for later ethylene determination. The root nodule complex was then removed from the jars and put into labeled plastic bags and frozen for later determination of nodule number and mass. A second harvest, following the same procedure, was conducted 53 DAP.

Time course study. In conjunction with the diurnal study, a field time course experiment was carried out using 4 high \( \text{N}_2 \) fixing genotypes ('H-Brown Crowder', 'H-Knuckle Purple Hull', 'H-Calico Crowder', 'H-TXCS 8') and 3 low \( \text{N}_2 \) fixing genotypes ('L-Chinese Red', 'L-TX 460', 'L-Bush Purple Hull') (9). The experimental design was a randomized block with 5 replications. The genotypes were planted on April 18, 1978, using the experimental procedure described in the previous study. Five plants of each genotype from each block were harvested at 23, 34, 46, 53, 62 and 79 DAP. All plants were harvested between 1100 and 1300 hr on each harvest date. Plant dry weight, nodule number, nodule mass and plant specific activity (PSA) (µmoles \( \text{C}_2\text{H}_4/\text{plant-hr} \)) were determined as previously described.

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Results

**Diurnal study.** Diurnal patterns of acetylene reduction were similar for both 'H-Brown Crowder' and 'L-Bush Purple Hull' on each sample date (Fig. 1, Table 1). Although there were significant differences between genotypes, both were actively fixing nitrogen by 0800 hr. Activity peaked at 1200 hr and then declined rapidly, with a minor peak at 2400 hr for 'H-Brown Crowder'. At 34 DAP, significant differences were found between genotypes for PSA, at the 0800, 1200, 1600, and 2000 hr sampling periods. At 53 DAP significant differences were found at 0800, 1200, and 2400 hr.

**Time course study.** Significant differences were found between harvest dates for all variables measured (Table 2). PSA ranged from 6.2 at 23 DAP to 69 at 46 DAP, then declined to less than 8 at 79 DAP. This 46 day peak in activity was accompanied by a peak in flowering in 'H-Brown Crowder', 'H-Calico Crowder', 'H-Knuckle Purple Hull', 'L-Chinese Red' and 'H-TXCS-8'. Both 'L-Bush Purple Hull' and 'L-TX 460' reached peak flower about 1 week earlier. A similar pattern was found for nodule mass, nodule number and plant dry wt.; however, these parameters peaked at 53 DAP.

Significant differences among genotypes were also found within harvests for all variables measured. For example, PSA (Fig. 2) at 23 DAP indicated that all genotypes were behaving in essentially the same manner. However, by 34 DAP, 'H-Calico Crowder' had significantly greater activity than other genotypes, and at 46 DAP all 4 high fixing genotypes were significantly more active. Two of the low fixing genotypes were beginning to decline in activity at this harvest. All genotypes had dropped in activity by 53 DAP, with 'H-Brown Crowder', 'H-Calico Crowder' and 'H-Knuckle Purple Hull' significantly more active than all other genotypes. These differences persisted through 62 DAP but had disappeared by 79 DAP.

**Discussion**

The diurnal patterns of nitrogen fixation in southernpea, for both high and low fixing genotypes, were very similar. Our acetylene reduction results indicated the presence of 2 peaks—one at 1200 and the other at 2400 hr, at both 34 and 53 DAP. These results are similar to those of Ayanaba and Lawson (1) who found 2 maxima, between 0600 — 1200 and 1800 — 2400 hr, and 2 minima, between 1200 — 1600 and 2400 — 0600 hr, at 55-56 DAP. Balandreau et al. (2), using peanuts, also found 2 peaks in activity, but these occurred at about 0600 and 1600 hr. The differences in these experiments could be attributed to different environmental conditions. Certainly, the rapid decrease in C2H2 reduction in our experiment from 1200 to 1600 hr could be attributed to the rapid increase in both soil and air temperatures, which peaked at 1600 hr (Fig. 3). Under the environmental conditions of this experiment, the optimum time of sampling for maximum rates of C2H2 reduction was between 1100 and 1300 hr.

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**Table 1.** The effect of genotype and growth stage on diurnal plant specific activity in southernpea.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hour</th>
<th>34 days</th>
<th>53 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bush Purple Hull</td>
<td>0800</td>
<td>3.0b</td>
<td>8.0cd</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>5.6a</td>
<td>31.9a</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>1.8c</td>
<td>23.7b</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>1.6c</td>
<td>10.8c</td>
</tr>
<tr>
<td></td>
<td>2400</td>
<td>1.8c</td>
<td>7.4cd</td>
</tr>
<tr>
<td></td>
<td>0400</td>
<td>1.7c</td>
<td>4.9d</td>
</tr>
<tr>
<td>Brown Crowder</td>
<td>0800</td>
<td>11.4b</td>
<td>57.3b</td>
</tr>
<tr>
<td></td>
<td>1200</td>
<td>22.0a</td>
<td>101.4a</td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>6.0c</td>
<td>27.2c</td>
</tr>
<tr>
<td></td>
<td>2000</td>
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<td>12.2d</td>
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<td>2400</td>
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<tr>
<td></td>
<td>0400</td>
<td>1.1d</td>
<td>14.2d</td>
</tr>
</tbody>
</table>

2Age in days after planting.

3Mean separation within columns by Duncan's multiple range test, 5% level.
Fig. 2. Effect of genotype and growth stage on plant specific activity in 4 high and 2 low N₂ fixing southernpea lines.

Results from the time course experiment indicated that sampling for maximum rates of C₂H₂ reduction should occur as the plants reach full flower. Thus, sampling may vary between genotypes depending on maturity class. These results closely parallel the work of Graham and Rosas (4) using *Phaseolus vulgaris*. They found that, regardless of plant type, maximum C₂H₂ reduction and nodule development occurred between the onset of flowering and the start of pod fill.

With diurnal and time course patterns of C₂H₂ reduction approximated, phenotypic selection of superior N₂ fixing southernpea genotypes can effectively take place.

Literature Cited