Evaluating Unique *Paspalum* Germplasm for Use in Nitrate Mitigation in the Suwannee River Basin

**Year 1 Report (7/01/2006 to 6/30/2006)**

To Florida Department of Agriculture and Consumer Services

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We proposed that *Paspalum* germplasm adapted to varying water inputs and selected for deep, vigorous rooting will be better than other subtropical grasses at removing nitrates via plant uptake, storage, and volatilization. During this first year of funding, we purchased critical farm and laboratory equipment, procured additional dedicated greenhouse space, established demonstration-size (> 1 acre) forage plots and smaller experimental field plots and established demonstration and research plots at two dairies. The following is a description of the past year's activities and preliminary findings.

**Greenhouse Activities**

Over the past year, an additional 55 m² of greenhouse space at the NFREC-Quincy has been allocated to forage BMP research. Contracts are in place to install deionized water and four 1000-watt high pressure sodium lamps for conducting forage fertilization/BMP studies year-round. These should be completed in August, 2006. Another 55 m² continues to be used for developing cultivars for use in BMP studies. Clonal propagation of several bahiagrass lines in the greenhouse continues in 2006.

**Fields Activities**

*Seed Increases*

**Bahiagrass.** The bahiagrass seed increase focused on new diploid and tetraploid germplasm. Diploid photoperiod and cold adapted (PCA) populations FL PCA Cycle 3, FL PCA Cycle 4 and FL PCA Cycle 5 were selected for early spring and late fall forage growth, and improved rooting structure. These cycles have been grown, alternating between Marianna and Ona FL, selecting over wide environments to improve cold tolerance and photoperiod response. In addition to those physiological traits, progress has been made in selecting for resistance to the dollar spot fungus, increasing rooting/stolon mass, and selecting for more rapid seed germination. FL PCA Cycle 3, FL PCA Cycle 4, and FL PCA Cycle 5 are currently in yield trials at the Range Cattle REC, Ona, the Coastal Plain Experiment Station, Tifton, the NFREC, Marianna and the Agronomy Forage Research Unit (Dairy Research Unit), Hague. FL PCA Cycle 4 is also being evaluated at Mississippi State University for cold tolerance. Breeder’s seed increases of FL PCA Cycle 3 and FL PCA Cycle 4 were planted at two locations in Florida in 2003 and acreage was increased at both locations in 2005. FL PCA Cycle 5 breeder’s seed increase was seeded in April 2006 at the NFREC-Marianna. Breeder’s seed of all cycles (FL PCA 3, 4, and 5) were harvested in June 2006 and should continue through August 2006 pending adequate rainfall and good seed production conditions.

Tetraploid or ‘Argentine-type’ bahiagrass evaluations and seed increases are also underway. Tetraploids tend to be very robust plants that germinate quickly and spread rapidly to cover new land within the first year of establishment. Breeding improvement has been limited with
‘Argentina’ and ‘Paraguay 22’ due to the chromosome number and type of reproduction (apomixis) in the plant. We recently imported new germplasm from Argentina to use in our tetraploid crossing program. In addition to the new acquisitions, we have been able to successfully use chemicals on ‘Tifton 9’ (a diploid type) to create new tetraploid lines. These lines have been crossed with the new material from Argentina and several other tetraploid types that have desirable features for use as a utility turf or forage. Experimental lines are being evaluated at the Agronomy Forage Research Unit (Dairy Research Unit), Hague and at the NFREC-Marianna. Several new hybrids appear to have desirable phenotypes and will be tested for rooting behavior and nutrient utilization in greenhouse pot studies and at the NFREC-Live Oak as field-grown plants. We anticipate superior genotypes will be further evaluated for potential variety releases.

**Annual ryegrass.** Two populations of annual ryegrass (FL MAR Early 4x and FL MAR 2x) were seed increased at the NFREC-Marianna in 2006. Both experimental lines have been developed for use on dairy operations for early winter forage silage. The FL MAR Early 4x was used in the "on-farm" dairy study we conducted in Suwannee Valley in 2006 (see on-farm activities).

**Small grains.** Small grains are another popular winter annual cover crop forage. Seed increases of new experimental oat lines were harvested in 2006. We anticipate one new oat variety (LA966-270) to be released from this program in 2006.

**NFREC demonstration/research strips**

Large (> 1 acre) strips serve as demonstration sites for forage variety comparisons and will also be used to conduct side-by-side comparisons of potential BMP practices, such as fertilization management strategies. The larger scale provides proof of concept of what is deduced from greenhouse and small plot studies.

**Bermudagrass.** Bermudagrass functions as our baseline forage to compare our bahiagrass progress in nutrient removal and effects on water quality. Four varieties of bermudagrass (Tifton 85, Coastal, Russell, and Alicia) were sprigged (approximately 1.5 acres per cultivar) in spring 2005 at the NFREC, Live Oak and established into swards during the 2005 growing season. These plantings have been fertilized in spring 2006 based on UF-IFAS recommendations. Plans are underway for 2006 to plant approximately one acres strips at the NFREC, Marianna to support multi-location comparisons.

**Bahiagrass.** Three varieties of bahiagrass (Tifton 9, Argentine and Pensacola) were seeded (approximately 2 acres per variety) in summer 2005 at the NFREC, Live Oak. Full swards were established by spring 2006. A seed increase of FL PCA Cycle 4 bahiagrass was harvested in early June 2006 at the Range Cattle REC to be used in establishing a 2-acre planting at the NFREC-Live Oak. Similar size strips were also planted at the NFREC, Marianna for multi-location comparison purposes in 2005. The FL PCA Cycle 4 bahiagrass will be seeded in summer, 2006.

**Experimental Plots.**

**Fertilizer management studies.** Six replicated (5 x 100 ft) strips of 4 different bahiagrass cultivars were established at the NFREC, Marianna in 2005 to support fertility work on bahiagrass varieties. The strips will be used in 2006 for factorial studies addressing fertilization application rates and timing.
Root characterization studies. Improving 'Pensacola' bahiagrass above-ground production via recurrent phenotypic selection procedure has had an unknown effect on rooting structure form and function. Bahiagrass cultivars (‘Tifton 9’, ‘Argentine’, ‘Paraguay 22’ and ‘Pensacola’) and two experimental breeding lines (RRPS Cycle 23 and Tifton 7) are being evaluated for rhizome-stolon mass, root mass, rhizome-stolon C:N ratio and nutrient uptake. Both, diploid and tetraploid lines are being investigated since ploidy level is believed to influence the size and possibly the capacity of the rhizome-stolons to store carbohydrates and nutrients. Bahiagrass genotypes were planted in 6 replicated (5 x 10 ft.) plots at the NFREC, Quincy, FL in 2002. Established plots were cored (10 cm x 10 cm) in 2004 to examine rhizome-stolon and root growth under high fertilization. They are currently being sampled again (10 cm x 122 cm depth) as established plants under low fertility. Following sampling, the plots will be fertilized according to IFAS recommendations and roots sampled in winter to determine changes in root growth and potential for nutrient uptake over time. Root mass and depth differences among bahiagrass genotypes will likely lead to differences in nitrogen and other nutrient transport (i.e., utilization, mineralization, and denitrification).

Increased microbial activity is associated with the rhizosphere, where activity is orders of magnitude greater than in bulk soil. Sufficient carbon (energy), moisture and electron donors (O2 or NO3 under anoxia) promote denitrification if there is an active microbial community. Microbial activity can be measured via respiration. A technique is being developed to test the potential soil respiration among different bahiagrass genotypes and fertilizer amendments in surface and subsoils using a rapid microtiter assay based on fluorometric detection of O2 consumption. To validate processing methods, soil cores (2.5 x 122 cm) were taken from the above-mentioned bahiagrass field plots and sampled to 122 cm depth. A preliminary test to validate methodology was completed in spring, 2006. Elevated substrate (i.e., carbon sources) induced respiration was observed to a depth of 46 cm. Response without supplementation was consistently observed in the upper 15 cm of soil. The addition of NH4NO3 with the substrate increased the response somewhat more than substrate alone. The seasonal respiration response of the different bahiagrass cultivars to IFAS fertilization recommendations will continue through the summer of 2006.

A screening procedure for bahiagrass root elongation (growth) rate was under development in 2005. A screening procedure will help to quickly identify new genotypes for favorable rooting traits. Dozens of genotypes can be assessed each year with minimal cost and labor. It is our contention that small (3.5 cm dia x 90 cm long) acrylic columns may essentially mimic bahiagrass rooting in an undisturbed soil profile. In theory, more robust rooting lines will be better at capturing nitrates and other nutrients before they leach beyond the root zone and greater rooting may be more conducive to low nutrient input systems. These columns were filled with soil from Live Oak in three layers to mimic the depth differences of undisturbed soil. In addition, a low bulk density soilless mix was also compared. The bulk densities for the three layers from surface to 3 feet were 1.55, 1.49, and 1.59 g cm-3, respectively. The bulk density of the soilless mix was 0.77 g cm-3. These columns were compared to larger columns (10 cm dia. x 90 cm long) containing the same rooting media. Apomictic bahiagrass varieties Argentine and Tifton-7 were seeded in 11 replicated 10 cm columns and 24 replicated 3.5 cm columns. Both Argentine and Tifton 7 bahiagrass varieties are noted for their large root systems but the Tifton 7 appears to have a much larger root system than Argentine or other Pensacola-derived varieties, i.e., Tifton-9. Field plots at Live Oak were seeded at the same time so that plant roots could be
harvested in situ at the termination of the column study. This will help determine if the columns (large or small) adequately assessed rooting potential in the field. Seed germination failed in both the field plots and the columns during our 2005 attempt and the study was replanted spring 2006. Drought conditions at Live Oak resulted in germination failure but we obtained unexpectedly interesting results in the column comparisons. The depth of the deepest root visible through the acrylic wall was recorded three times a week starting immediately after germination.

Preliminary results showed differences between cultivars in the rate of root development for the initial phase (first 8 days after germination). Tifton 7 had more rapid germination and higher initial vigor, with root growth rate (RGR) averaging 2.5 cm d\(^{-1}\), while Argentine roots extended 1.9 cm d\(^{-1}\) or 24\% lower. However, these differences were not detected between day 8 and 27 days after germination; where roots from both cultivars extended approximately 2.3 cm d\(^{-1}\). Tifton 7 showed deeper roots on day 27 likely because of its higher initial vigor. RGR was higher for plants growing in larger columns (10-cm dia.). These differences were more evident at the initial phase of the experiment. Plant roots in 10 cm columns extended an average of 3.3 cm d\(^{-1}\) within the first 8 days, while plants growing in the 3.5 cm columns extended only 1.7 cm d\(^{-1}\) or 50\% slower. Smaller differences were detected between day 8 and 27 days, where 10 cm column rates were 2.5 cm day\(^{-1}\) and 3.5 cm dia. columns were 2.3 cm d\(^{-1}\). Root growth rates were greater for plants growing in soil, especially near the beginning of the experiment. An average elongation rate of 2.8 cm d\(^{-1}\) was recorded for plants in soil, versus 1.6 cm d\(^{-1}\) (or 43\% less) for plants in soilless mix, during the first 8 days. Over time, the differences decreased, with soil columns averaging 2.4 cm d\(^{-1}\) and soilless mix columns averaging 2.3 cm d\(^{-1}\). The study will be repeated during the summer, 2006 with an additional variable related to bahiagrass establishment. One variable to test is irrigation frequency. Droughty conditions are common during the typical sowing period (spring) for warm-season grasses in Florida. Removal or shading the photosynthetically active organs is also being considered. Early mowing is used during bahiagrass establishment for weed control, since the establishing plants are intolerant of many herbicides. Data from these studies will provide selection criteria to develop genotypes that can more rapidly reach soil moisture during establishment and better survive early establishment mowing. Quicker establishment will lead to less input losses to the environment and the trend of vigorous rooting genotypes will likely carry over into establish swards.

**On-Farm Activities**

On-farm activities are a combination of research and demonstrations used to test proof of concept of what has been developed at smaller and more highly controlled scales. Concurrently, new insights and challenges present themselves that provide feedback for improving our experimental studies. Although bahiagrass is not in high demand by dairies, winter forages are. Bahiagrass can be successfully over-seeded with many of the same winter annuals that dairies demand. Therefore, we are developing high nutrient removing winter annual selections and management strategies that also can be incorporated into year-round bahiagrass-based forage systems. Dairies are a good proving ground for forages for that should meet nutrient removal demands.

**Dairy Research Unit**

Annual ryegrass. Demonstrations of three annual ryegrass varieties (Diamond R and Jumbo) and an experimental line (FL MAR Early 4x) were grown for silage at the Dairy Research Unit, Hague. Visual ratings on early growth and seasonal forage production were made. FL Mar Early
4x produced earlier tonnage, had excellent vigor and continued to provide long-season production compared with other varieties tested.

**Triticale.** A demonstration of forage triticale (Monarch) grown for silage was conducted at the Dairy Research Unit, Hague. Visual ratings on early growth and seasonal forage production were made. Monarch produced good forage yield in a late planted situation and may be an alternative forage silage crop for use at local dairies.

**Commercial Dairy**
Dairy nitrate contributions to surface and groundwater have been an area of focus over the past several years in Florida. Improved crop management may reduce N leaching losses. For example, annual ryegrass is typically used as a N catch crop during the winter. Additionally, greater forage production results in greater N removal. Nutrient removal can be improved by increasing productivity (more nutrient removed by the plant) and nutrient interception (improved root systems), particularly in subsoils. Tetraploid bahiagrass cultivars tend to have more vigorous and massive root systems than do diploid cultivars. However, no reports on ploidy-level effects on root development and nutrient removal by annual ryegrass are known. The following three annual ryegrasses were compared in winter, 2006:

<table>
<thead>
<tr>
<th>Tetraploid</th>
<th>Diploid</th>
</tr>
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<tbody>
<tr>
<td>Jumbo (JUM)</td>
<td>Marshall (MAR)</td>
</tr>
<tr>
<td>FL X1998 (New) 4X late (E4X)</td>
<td></td>
</tr>
</tbody>
</table>

These cultivars were seeded on 10 x 40 ft plots replicated 4 times (n = 12) under dairy effluent irrigation pivots (either standard overhead spray or drop nozzles) in an old (in production over 4 years) and a new (in production one year) fields at a commercial dairy in Suwannee county. A mixed repeated measures model was used to analyze data (SAS9.0, Cary, North Carolina). Due to inclement weather, seeding was delayed so only 2 harvests occurred. Typically 3 harvests can be achieved. In 2006, yield, tissue content and available soil nutrients (0 – 46 cm) were compared among cultivars, nozzle types and field ages. Forage quality and soil N is currently being analyzed.

Forage yields were greatest with the Early 4x (Fig. 1). This cultivar was developed for earlier production and improved disease resistance. However, it outperformed the mid to late season varieties, even at the end of the season. The commonly grown Marshall, had rapid yield declines from March to May, in part, from increased susceptibility to leaf spot disease. Dairies are particularly susceptible to leaf diseases because of high water input via irrigation.

Plants in the new field produced approximately 14% more tonnage than plants in the old field. Across all annual ryegrass cultivars, the worst treatment combination was drop nozzle irrigation in the old field. The drop nozzles were located at the last pivot span. It is possible that water application rates or quality was different in the last span. These factors will be monitored when the study is repeated in 2007. Extractable soil Mg, S and B were greater in soils associated with the drop nozzles while extractable Mn was greater in soils with the standard nozzles. Considering the drop nozzles have not been in use long, these soil differences may be more due to soil spatial differences than nozzle placement. The irrigation water will be tested for mineral content, since this may be a source of additional Mg and S.
As expected, the new and old fields differed with some soil parameters. Nutrients had greater stratification in the old field since lagoon effluent was in use much longer (> 4 years) than in the new field. Unlike many of the soils in Suwannee county, this dairy has a heavier textured subsoil that lessens nutrient leaching. The old field soil had greater extractable P, K, Zn, Cu and Mn than the new field, while the new field had greater Fe and B, in general (Table 1). Calcium, Mg and S were not different between fields or with soil depth from 0 to 46 cm. From 0 – 15 cm, extractable soil P was 169 mg kg⁻¹ in the old field but values fell to 55 mg kg⁻¹ at 15 – 30 cm. At 30 – 46 cm, soil P was 17 mg kg⁻¹, which was comparable to P values from the new field. The top soil (0 to 6 cm) appears to be accumulating P but the lower depths are far from saturated. Extractable soil Cu was nearly 5 times greater in the old field topsoil than the new field. As the depth increased, soil Cu in the old filed declined until it was comparable to the new field at the 30 – 46 cm depth. Zinc also was higher in the old field topsoil. Although K, Mn, Fe, and B were different between fields, the values were not of concern in regards to plant nutrition or environmental quality.

Forage tissue P and K were greater in March than May, while tissue Ca, B and Mn were greater in May. There were no harvest period differences for the other nutrients. Differences in soil composition did not appear to affect tissue nutrient concentrations but there was a cultivar difference for tissue P. Jumbo had approximately 9% greater tissue P than Marshall. Using the yield and tissue content values, the nutrient removal from soil was calculated (Table 2). Early 4x removed twice as much nutrients as Marshall and 30% more nutrients than Jumbo. Data is being analyzed for N but even if tissue N content is shown to be equivalent among cultivars, N removal will be greater for Early 4x, based on the greater forage yield. The study will be repeated in Winter, 2007.

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Fig. 1. Annual ryegrass yields from winter, 2006. Vertical bars = standard errors.
### Table 1. Soil nutrient composition at harvest, May, 2006.

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth</th>
<th>pH</th>
<th>OM† (g kg$^{-1}$)</th>
<th>EC‡ (µS)</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>B</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>New</td>
<td>0 - 15</td>
<td>5.55</td>
<td>28.4</td>
<td>161</td>
<td>37</td>
<td>111</td>
<td>703</td>
<td>143</td>
<td>17</td>
<td>0.30</td>
<td>172</td>
<td>13</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>15 - 30</td>
<td>5.37</td>
<td>23.3</td>
<td>69</td>
<td>21</td>
<td>42</td>
<td>541</td>
<td>96</td>
<td>18</td>
<td>0.21</td>
<td>151</td>
<td>11</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>30 - 46</td>
<td>5.09</td>
<td>31.5</td>
<td>58</td>
<td>11</td>
<td>41</td>
<td>683</td>
<td>114</td>
<td>27</td>
<td>0.22</td>
<td>112</td>
<td>8</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Old</td>
<td>0 - 15</td>
<td>6.52</td>
<td>24.0</td>
<td>166</td>
<td>169</td>
<td>113</td>
<td>991</td>
<td>144</td>
<td>16</td>
<td>0.29</td>
<td>124</td>
<td>25</td>
<td>6.1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>15 - 30</td>
<td>6.31</td>
<td>18.5</td>
<td>78</td>
<td>55</td>
<td>66</td>
<td>576</td>
<td>120</td>
<td>16</td>
<td>0.17</td>
<td>82</td>
<td>14</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>30 - 46</td>
<td>6.15</td>
<td>20.9</td>
<td>68</td>
<td>17</td>
<td>64</td>
<td>450</td>
<td>114</td>
<td>19</td>
<td>0.13</td>
<td>60</td>
<td>10</td>
<td>0.7</td>
<td>0.3</td>
</tr>
</tbody>
</table>

†OM = organic matter using the loss-on-ignition method.
‡EC = electrical conductivity (an assessment of salt or nutrient loading in the soil).

### Table 2. Nutrient removal by 3 annual ryegrass cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>B</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early 4x</td>
<td>52</td>
<td>503</td>
<td>51</td>
<td>26</td>
<td>28</td>
<td>0.07</td>
<td>1.22</td>
<td>0.85</td>
<td>0.52</td>
<td>0.14</td>
</tr>
<tr>
<td>Jumbo</td>
<td>39</td>
<td>360</td>
<td>35</td>
<td>18</td>
<td>18</td>
<td>0.05</td>
<td>0.89</td>
<td>0.62</td>
<td>0.37</td>
<td>011</td>
</tr>
<tr>
<td>Marshall</td>
<td>24</td>
<td>206</td>
<td>22</td>
<td>11</td>
<td>11</td>
<td>0.03</td>
<td>0.60</td>
<td>0.37</td>
<td>0.23</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Field Days and Workshops

*Florida’s State Forage Workers Tour (August, 2005)*
Producers, county and state IFAS faculty toured forage producers in Suwannee and Madison county, and the forage field work at NFREC, Live Oak on the first day. They spent the second day at the NFREC, Marianna, where we discussed on-going forage research.

*North Florida Beef and Forage Field Day (August, 2005)*
Producers and county IFAS faculty participated in a tour of the NFREC, Marianna. Tour presentations included bahiagrass breeding lines and the importance of forage BMPs in North Florida.

*Row Crop/Vegetable BMP Manual Training (September, 2005)*
County IFAS faculty participated in a BMP in-service class to help with disseminating BMPs. A presentation on interpreting forage BMPs was given.

*Fall Forage Production Meeting (October, 2005)*
Producers attended an on-farm field day event. Presentations on winter annual forage selection and management and on forage soil fertility management.

*Agronomy in-service (January, 2006)*
This event provided updates on issues county faculty may come across over the upcoming season regarding commodities, including forages. A presentation on fertilizer use issues was given.

*Beef Cattle Short Course (May, 2006)*
Producers, state and county faculty attended this event. A presentation on bahiagrass fertilizer management was given.

* Emerging Trends in Pasture Fertilization (May, 2006)*
County and state faculty attended an in-service focused on pasture BMP-related issues. We organized this in-service and a presentation on forage BMPs was given.