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Developing and Validating a New Method to Improve Breeding for Cold-tolerant Bermudagrass

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Objectives: To develop a new technique that simplifies evaluation of bermudagrass for cold tolerance, and thereby improves breeding efficiency and facilitate the process.

In 2015, we have been working on the segregating population, provided by co-PI Wu. This segregation population was made by crossing a cold-tolerant entry A12396 (pollen donor) with a cold-sensitive entry A12395 (seed parent). The entry A12396 is a breeding line selected from the Oklahoma State University (OSU) bermudagrass germplasm known for cold hardiness. The entry A12395 is a collection from Puerto Rico which is susceptible to low temperature stress. With such a cross, we generated a population with anticipated segregation for cold tolerance.

First, a replicated experiment was conducted to evaluate the parent plants for their tolerance to AOPP herbicide. Results showed that A12395, the cold-sensitive entry collected from Puerto Rico, exhibited significant susceptibility to the AOPP herbicide, compared to the cold-tolerant entry A12396 (Fig 1). After germinating the progenies, the first set of 30 progenies were subjected to herbicide application in a replicated experiment. Data showed a differential response to the herbicide as we hypothesized (Fig 2.). As of now, we have screened 60 of a total 120 germinated progenies from this population. The current plan is to continue screening, then select representative progenies that exhibit various levels of herbicide tolerance for a chilling stress experiment. The correlation of plants to the two stresses will then be evaluated, before planting the selected plants into the field in the spring of 2016.

The second experiment we conducted was to rule out the possibility that the herbicide tolerance could be due to differences in herbicide absorption and/or translocation. A ^{14}C-labeled fluazifop (Fusillade II) has been acquired from Syngenta, and a known cold-tolerant and a cold-susceptible bermudagrass have been selected to perform such an experiment (Fig 3). All experiments were carried out with 3 replications. The isotope-labeled experiment revealed that, between the cold-tolerant and cold-susceptible bermudagrass plants, there were no or minimal differences in AOPP herbicide absorption (Fig 4) and translocation (Fig 5). These results collectively suggest other mechanisms, such as metabolism, especially in the fatty acid biosynthesis pathway, might contribute to the observed differential responses, and possibly explains the observed correlation between bermudagrass plant's responses to cold and AOPP herbicide. Currently, lipid fatty acid profiling is ongoing, which could potentially shed light as to the possible mechanism.

The future plan for this study in 2016 is therefore, to conduct a field experiment regarding the segregation progenies, and continue the lipid fatty acid profiling experiments to search for a possible mechanism.

Summary:

- A segregation population, based on the cold tolerance, has been screened and shown anticipated responses to AOPP herbicide;
- An isotope-labeled experiment has ruled out the possibility that differential responses were due to herbicide absorption and/or transportation;
- Future experiments are ongoing to decipher the underlying mechanism, possibly related to lipid fatty acid biosynthesis.

Fig 1. Bermudagrass responses to AOPP herbicide Acclaim Extra® (a.i. fenoxaprop-ethyl), at 0, 11, 18, and 25 days after treatment application. The top four pictures are entry A12395, a cold-sensitive collection, and the bottom four pictures are entry A12396, a cold-tolerant selection. In each picture, the top four pots were plants that received herbicide application, and the pot at the bottom serves as a control without herbicide application.

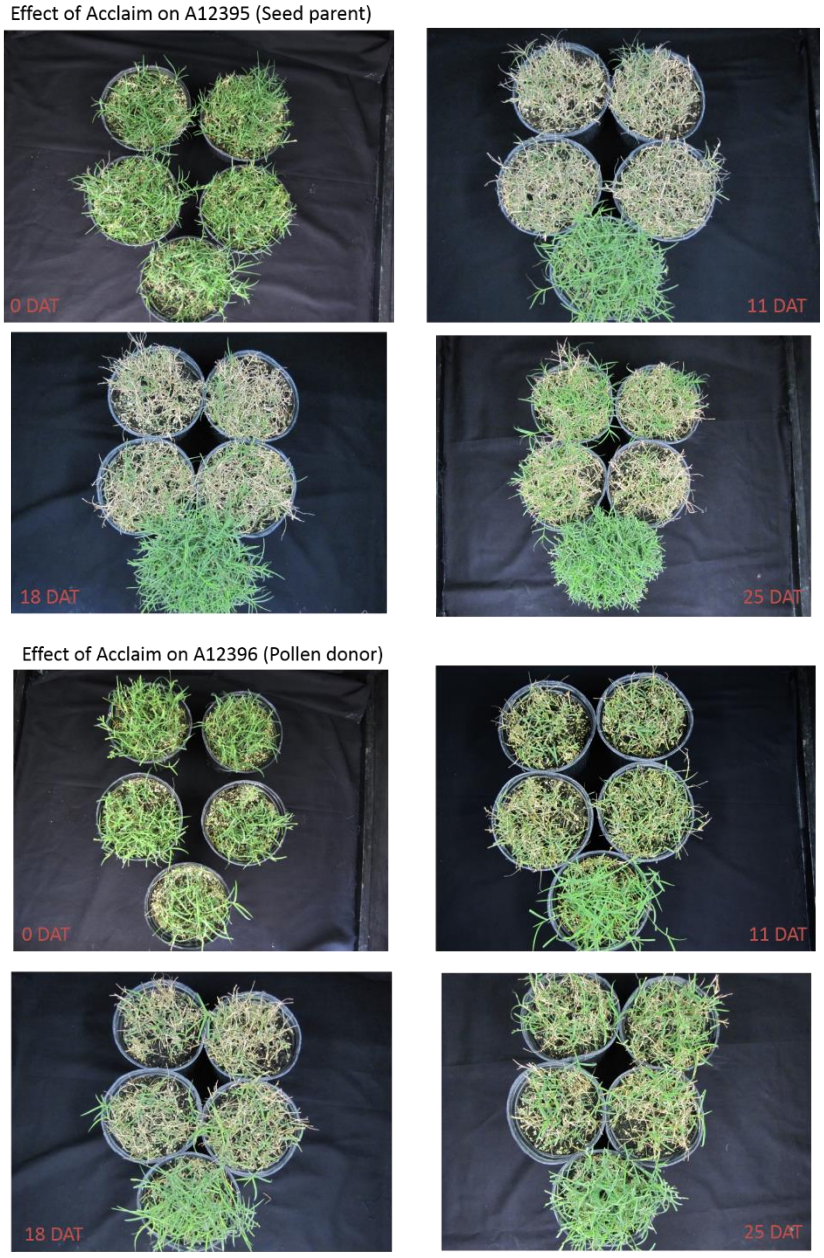

Fig 2. Representative images of progeny plants in response to Acclaim Extra® ((a.i. fenoxaprop-ethyl) application at 1 week after treatment.

Grasses resistance to Acclaim

Grasses susceptible to Acclaim

Fig 3. Bermudagrass plants prior to application of cold AOPP herbicide. Note the designated leaf that will be treated with isotope-labeled AOPP herbicide was carefully covered.

Fig 4. Isotope-labeled herbicide expressed as percent of applied (%) in leaf wash (LW), total absorbed and recovered from 'Celebration' and 'Riviera' bermudagrass (*C. dactylon*). Bars labeled with the same letter were not significantly different based on Fisher's Protected LSD at $P<0.05$.

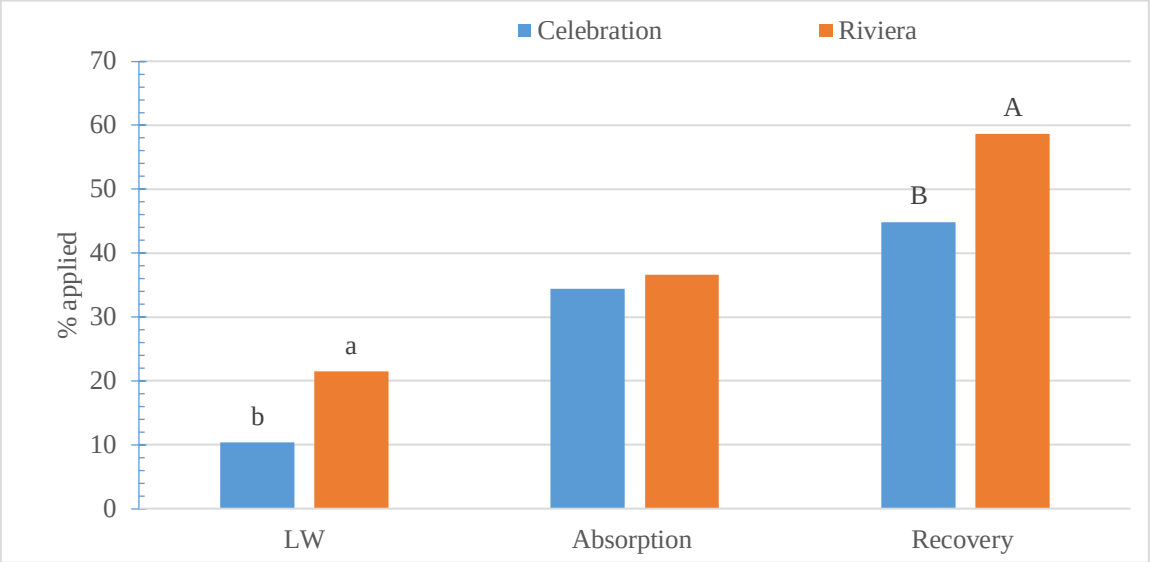

Fig 5. Isotope-labeled herbicide expressed as percent of applied (%) in treated leaf (TL), shoot above the treated leaf (SATL), shoot below the treated leaf (SBTL), rhizome and roots from 'Celebration' and 'Riviera' bermudagrass (*C. dactylon*). Bars labeled with the same letter were not significantly different based on Fisher's Protected LSD at $P<0.05$.