Cross-Protection against ALS-Inhibiting Herbicides in Non-Transgenic Switchgrass Selected for Imazapic Resistance

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Abstract
Switchgrass (*Panicum virgatum L.)* is a North American native warm-season perennial grass that is a cornerstone species in conservation systems and can also be utilized as a high-quality forage. 'Tusca' is a cultivar of lowland switchgrass selected from 'Alamo' (USDA NRCS, Knox City, TX) for resistance to the herbicide imazapic. This study was conducted to determine if selection in Tusca conferred cross-protection to other ALS-inhibiting herbicides. Five ALS-inhibiting herbicides, including imazapic (IPIC), imazamox (IMOX), imazapyr (IPYR), imazethapyr (ITHR), and metsulfuron methyl (MSUL), were tested on Tusca, Alamo, and wildtype johnsongrass (*Sorghum halepense* L. (Pers.)) at five rates (25, 50, 75, 100, and 125% of the recommended label rate) plus an untreated control, under laboratory (PRE) and greenhouse (POST) conditions. Alamo was used as a negative control and johnsongrass was used as a reference species to confirm efficacy of herbicide treatments. Six replications of 25 seed of both switchgrass cultivars and johnsongrass were screened for response to herbicide treatment at germination, as well as the 3-leaf stage. Mean germination percentage for untreated Tusca, Alamo, and johnsongrass were 71.5, 24.8, and 40.8, respectively. Compared to controls, mean germination percentage of Tusca remained >50% at all rates of ITHR and IMOX. Alamo exposed to ITHR had <25% germination and <50% for IMOX. While Tusca shows some improved resistance to IPIC at germination, greater resistance was found to ITHR, IPYR, and IMOX, whereas mean germination percentage of Alamo was significantly reduced by all treatments.

Introduction
In North America, switchgrass (*Panicum virgatum L.*) is a native warm-season perennial grass found across most of the country apart from the western states (Ball et al. 2015). It is utilized for conservation systems, wildlife habitat, erosion control, renewable bioenergy, and can be a high-quality forage in the spring and early summer. It spreads by short rhizomes and has a deep, fibrous root system. The two cultivars used in this study were lowland ecotypes which grow to a height of 3 to 6 feet tall, as opposed to upland species which grow to a height of 1 to 3 feet tall. The seed head is a wide panicle producing around 400,000 seeds per pound (Vandevender 2009). Switchgrass is cross-pollinated and highly self-incompatible (De Leon 2005). 'Tusca' is a cultivar of lowland switchgrass selected from 'Alamo' (USDA NRCS, Knox City, TX) for tolerance to the ALS-inhibiting herbicide imazapic. In prior studies, Tusca has shown significantly increased tolerance to imazapic when field-applied to established stands. Given that Tusca is the result of recurrent phenotypic selection of a cross-pollinated species, the opportunity for increased tolerance to herbicides from the same chemical family via inadvertent selection are possible (Tal 2000). In applications where switchgrass is planted in polyculture with other native plant species, almost all relevant native grasses and forbs are naturally tolerant of imazapic, but switchgrass is not. The development of a switchgrass cultivar with tolerance to chemicals commonly used in right-of-way, forestry, and industrial management settings could assuage the concerns of landowners when considering including switchgrass in diverse, multi-species plantings.

Methods and Study Site
The PRE-bioassay experiment was conducted in the Department of Plant and Soil Sciences at Mississippi State University and the POST bioassay experiment at the R. R. Foil Plant Science Research Center in Starkville, MS. Methodology for this study was taken from literature published by Burgos 2015 and Beckie 2000. In the PRE-bioassay, for each herbicide, a stock solution of 25, 50, 75, 100, and 125% of the recommended label rate was used to make a 1% agar mixture. Six replications of 25 seed of Tusca, Alamo, and johnsongrass were placed in petri dishes and treated with every herbicide/rate combination. Petri dishes received 50 ml of agar/herbicide mixture. Seeds were placed on agar and maintained in an artificial environment chamber at 30° /20° C under long day conditions. Germinated seeds were counted every two days for two weeks. In the POST bioassay, seedlings of each species were grown in a greenhouse in 50-cell plastic trays and two replications of 15 seedlings of each species were treated with every herbicide/rate combination. Treatments were applied using a CO_2 powered handheld sprayer when seedlings reached the three to four-leaf stage. Injury was rated

on a scale of 1-5. Ratings and seedling mortality were recorded every seven days for 28 days. Injury ratings were defined as 1: <20% of seedlings show injury 2: <40% of seedlings show injury 3: <60% of seedlings show injury 4: <80% of seedlings show injury 5: >80% of seedlings show injury. Individual replications were harvested to determine biomass accumulation after 28 days.

Results

PRE-Bioassay

Tusca showed tolerance to IPYR, IMOX, and ITHR, as well as improved tolerance to IPIC. All rates of IPIC ($P<0.0001$), ITHR ($P<0.0001$), and IMOX ($P<0.0001$) significantly decreased germination of Alamo. There was a significant negative effect on germination of Alamo by IPYR ($P=0.0030$), and MSUL ($P=0.0425$) but only at 100 and 125% rates. Following PRE-Bioassay, root and shoot lengths were assessed for all germinated seedlings (data not shown). All rates of all herbicides significantly decreased root ($P<0.0001$) and shoot ($P<0.0001$) lengths of Alamo and wild-type johnsongrass, while Tusca showed decreased response to IMOX, IPIC, IPYR, ITHR and MSUL.

POST Bioassay

Compared to Alamo, Tusca exhibited improved tolerance to IPIC, IPYR, IMOX, and ITHR at all rates applied (Figures 1&2). Injury of Alamo was extensive following treatment with IPYR, IMOX, ITHR, and IPIC. Wild-type johnsongrass proved to be a dependable bioassay marker for effectiveness of herbicide screening.

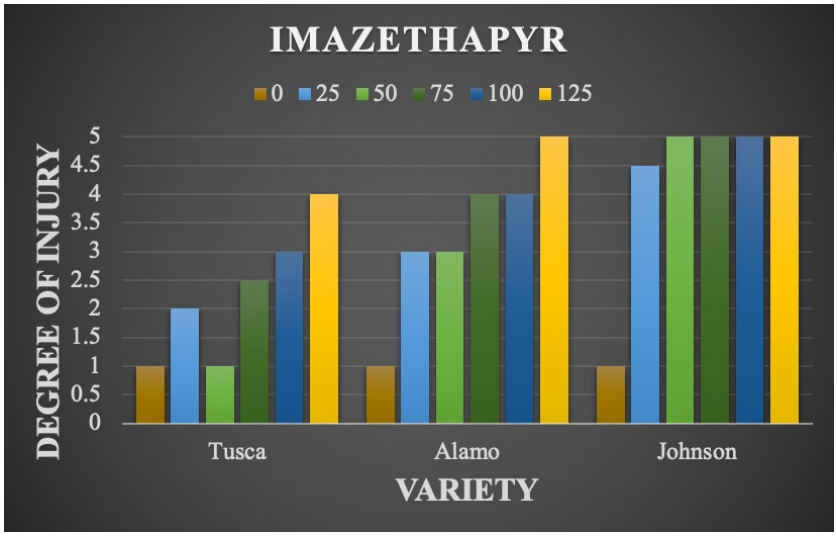

Figure 1. Mean injury ratings of two switchgrass varieties and wild-type johnsongrass taken 28-d after treatment was applied at the three to four leaf stage with imazethapyr herbicide at five rates.

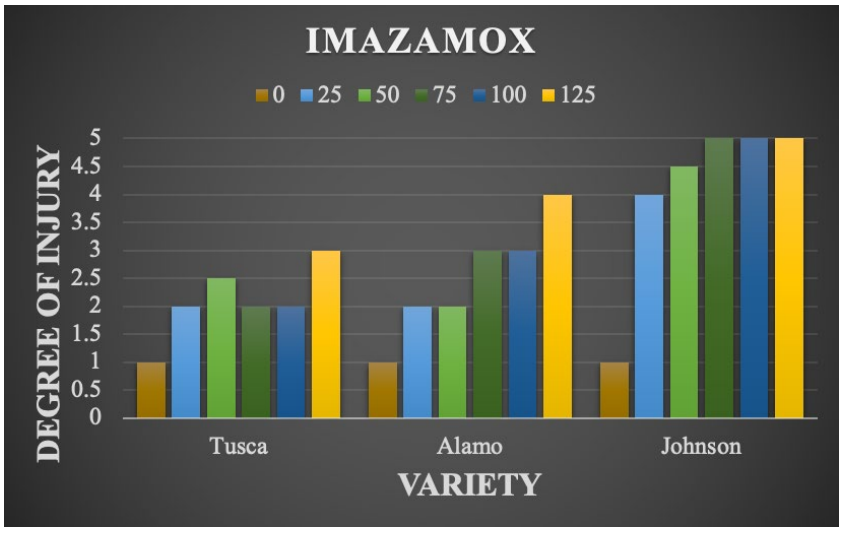

Figure 2. Mean injury ratings of two switchgrass varieties and wild-type johnsongrass taken 28-d after treatment was applied at the three to four leaf stage with imazamox herbicide at five rates.

Discussion [Conclusions/Implications]

Through inadvertent selection, Tusca also exhibits increased tolerance to other ALS-inhibiting herbicides at both PRE and POST application stage. With continued selection, Tusca can be improved for use in specific applications where these herbicides are commonly used. Tusca can be incorporated into polycultures and grasslands where this class of herbicides is commonly applied and imazapic can be added into the chemical rotation. This is of great value to land managers to prohibit or slow down any form of resistance among the population from repeated use of the same herbicide chemistry over time.

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