A regional monitoring program for herbicide resistance in shattercane and johnsongrass following commercialization of 'Inzen' sorghum hybrids

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INTRODUCTION

- 'Inzen' sorghum hybrids are resistant to ALS-inhibitor herbicide chemistries and are set to be released by DuPont-Pioneer in the near future.
- Mutations conferring ALS-resistance (ALS-R) in *Sorghum* weedy relatives shattercane and johnsongrass are neutral, and have as a result persisted in weedy populations since the mid 1990's.
- Inevitably, ALS-R alleles from Inzen will transfer to weedy populations throughout southern Nebraska and northern Kansas.
- Newly-introduced ALS-R mutations from Inzen, coupled with existing ALS-R mutations, will be selected for by herbicide applications and cause management challenges in the future.
- Here we propose a risk-assessment framework of ALS-R evolution in shattercane and johnsongrass to be used in a regional monitoring program in Nebraska and Kansas.

RATIONALE

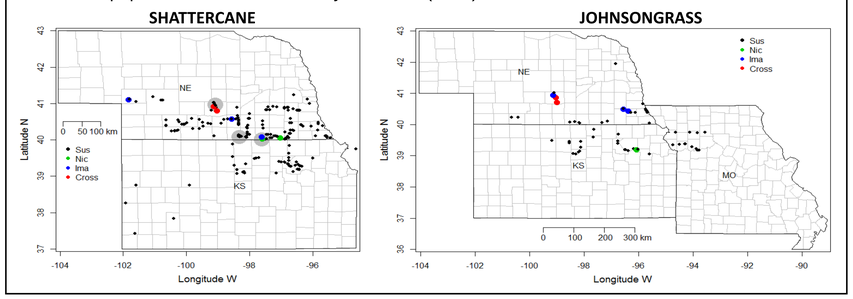
- Environmental impacts documented from Inzen commercialization can inform responsible deployment of future nuclear traits in sorghum.
- Identifying best-management practices to delay ALS-R evolution and minimize population densities of ALS-R weeds will prolong the lifespan of Inzen technology.

HYPOTHESIS

- Integrated investigations of population genomics and GxExM effects on weed reproductive biology can be used to minimize environmental effects of nuclear technology in sorghum.

MATERIALS AND METHODS

Figure 1. Werle et al. (2016) sampled >200 populations of weedy *Sorghum* in the Midwest. Sus = population susceptible to ALS herbicides tested, Nic = resistant to nicosulfuron, Ima = resistant to imazethapyr, and Cross = cross-resistant to both. The three gray circles denote areas where ALS-R shattercane populations were detected by Lee et al. (1999).



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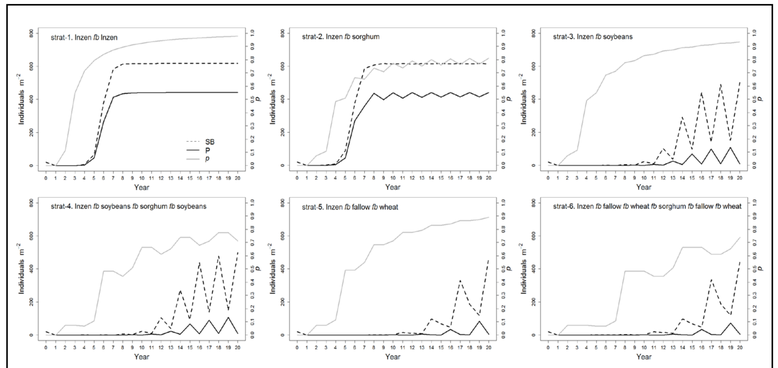

Figure 2. Total number of established shattercane plants m^{-2} (P) and viable seeds in the seedbank m^{-2} (SB), and frequency of the R allele (*p*) in the population at census over time, estimated by our deterministic model for each management strategy (strat-) considered. Best R management (strat-5) uses herbicide and crop rotation to maximize control in non-Inzen years.

Johnsongrass models are currently being developed. They are more complex because johnsongrass has rhizomes, is a perennial, and is tetraploid; whereas sorghum and shattercane are annual diploids.

Table 1. Identified target-site mutations. Wild-type codons at 560 and 574 encode Val and Trp, respectively. Leaf samples (2 resistant and 1 susceptible) were collected for each population. DNA was extracted and ALS gene sequence was PCR-amplified using the primers (F: 5'-GCAGTGGTTGTCTTCAGCTGGT-3'; R: 5'-GATCATAGGCAACACATGATCCT-3').

Population	County, State	Phenotype	Codon 560	Codon 574
INZEN HYRBIDS		Resistant (Ima + Nic)	**Ile**	**Leu**
		SHATTERCANE		
S-13	Lincoln, KS	Susceptible	Val	Trp
S-46	Adams, NE	Resistant (Ima)	Val	Trp
		Susceptible	Val	Trp
S-58	Buffalo, NE	Resistant (Ima + Nic)	Val	Trp
		Susceptible	Val	Trp
S-63	Buffalo, NE	Resistant (Ima + Nic)	Val	Trp
		Susceptible	**Ile**	Trp
S-105	Jefferson, NE	Resistant (Nic)	**Ile**	Trp
		Susceptible	**Ile**	Trp
S-117	Keith, NE	Resistant (Ima)	Val	Trp
		Susceptible	Val	Trp
S-125	Lancaster, NE	Susceptible	**Ile**	Trp
S-130	Lincoln, NE	Susceptible	Val	Trp
S-178	Thayer, NE	Resistant (Nic)	Val	Trp
		Susceptible	Val	Trp
S-179	Thayer, NE	Resistant (Ima)	**Ile**	Trp
		Susceptible	Val	Trp
		JOHNSONGRASS		
J-14	Pottawatomie, KS	Susceptible	Val	Trp
J-15	Pottawatomie, KS	Resistant (Nic)	Val	Trp
		Susceptible	Val	Trp
J-25	Caldwell, MO	Susceptible	Val	Trp
J-35	Buffalo, NE	Resistant (Ima + Nic)	**Ile**/Val*	Trp
		Susceptible	**Ile**/Val	Trp
J-36	Buffalo, NE	Resistant (Ima + Nic)	Val	**Leu**/Trp
		Susceptible	Val	Trp
J-37	Buffalo, NE	Resistant (Ima)	Val	Trp
		Susceptible	Val	Trp
J-40	Gage, NE	Resistant (Ima)	Val	Trp
		Susceptible	Val	Trp
J-44	Johnson, NE	Resistant (Ima)	Val	Trp
		Susceptible	Val	Trp
J-52	Nuckolls, NE	Susceptible	**Ile**/Val	Trp

*Not all resistant or susceptible plants in the population had the same amino acid substitution.

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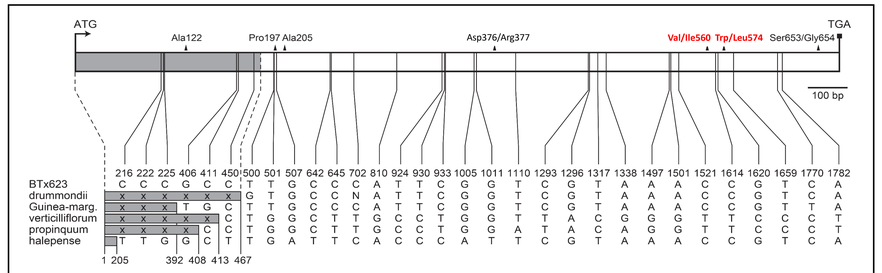
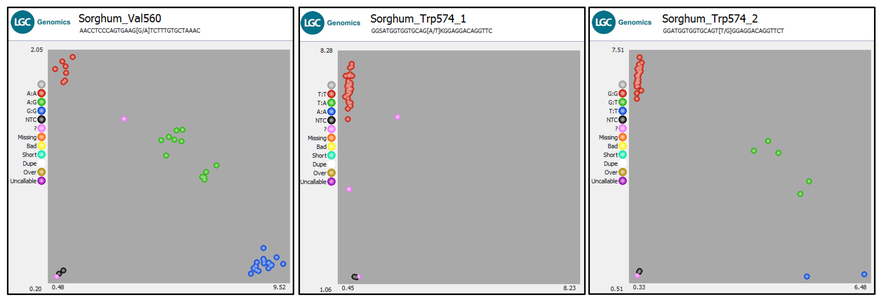


Figure 4. A set of KASP assays (table below) were developed (LGC Genomics) to target the two SNPs within the *ALS* target site gene sequence of Inzen. These will be used to **preliminarily screen plants** that survive ALS-inhibitor (Nic and Ima) application at 1.5X the field rate. Plants that do not have both Inzen SNPs will be considered ALS-R due to *de novo* mutation, not gene flow. Plants with both Inzen SNPs (red) will be screened for additional SNPs and indels (gray regions in the figure) unique to Inzen at the *ALS* locus to **confirm gene flow**, based on our recent assembly of *ALS* in *Sorghum* (Werle et al. 2016). These markers are currently being developed.

SNP ID	Sequence
Sorghum_Val560	AACCTCCCAGTGAAG**[G/A]**TCTTTGTGCTAAAC
Sorghum_Trp574_1	GGSATGGTGGTGCAG**[A/T]**KGGAGGACAGGTTC
Sorghum_Trp574_2	GGATGGTGGTGCAGW**[T/G]**GGAGGACAGGTTCT



Figures 5-7. Preliminary results (LGC Genomics) of each KASP assay targeting the ALS 560/574 SNPs unique to Inzen. Assay *Sorghum_Val560* detects a codon change from Valine to Isoleucine. Assay *Sorghum_Trp574_1* detects a codon change from Tryptophan to Methionine. Assay *Sorghum_Trp574_2* detects a codon change from Tryptophan to Leucine. *Val/Ile560* does not confer ALS-resistance, while *Trp/Met574* and *Trp/Ile574* both confer resistance.

FUTURE DIRECTIONS

- Develop a regional network of collaborators for the dissemination of grower surveys in 2017 to identify a baseline idea of where ALS-R shattercane and johnsongrass are currently located.
- Discuss the special challenge of crop-to-weed gene flow and the transfer of herbicide resistance to shattercane and johnsongrass.
- Advertise monitoring program and no-cost population sampling at extension meetings.
- Conduct follow-up surveys after three, five, seven, and ten years to track agroecosystem choice dynamics in response to available herbicide-resistant technology, and the rate of ALS-R evolution.
- Identify populations of interest to use in detailed molecular ecology analysis to study cropping system effects on weed invasiveness, competitive ability, genetic diversity, seedbank dynamics, rate of ALS-R allele appearance and diversity, etc.
- Generate quantitative data on the relative rates of ALS-R evolution due to gene flow vs. herbicide selection in shattercane and johnsongrass.