Herbicide Resistant Systems for Turfgrasses

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ABSTRACT

Herbicide resistance has been a sought-after trait to improve weed control options in turfgrasses; however attempts to commercialize genetically modified (GM) turfgrasses have been unsuccessful. Sethoxydim is a grass-specific herbicide in the ACCase family. Naturally occurring resistance to sethoxydim and other ACCase herbicides has been reported in several grass species as a result of single base-pair mutations. Research was initiated to develop a novel source of resistance to sethoxydim in seashore paspalum (Paspalum vaginatum, Swartz). In vitro selection and regeneration using tissue culture were used to select for naturally occurring mutations conferring herbicide resistance. Callus was induced from immature inflorescences then plated on callus induction medium containing 10 µM sethoxydim for selection. Green plants were regenerated from resistant callus, the Ile to Leu mutation known to confer sethoxydim resistance was documented, and expression of herbicide resistance confirmed. New sethoxydim resistant seashore paspalum experimental lines are now under field evaluation to determine their potential as a new tool to manage bermudagrass and other problematic grasses in seashore paspalum turf.

INTRODUCTION

Herbicide resistance is a trait that has been actively pursued for many crops to improve the efficiency of weed control and as a means to control problematic weeds. Arguably the most notable herbicide resistant systems are the Roundup Ready® (RR) products. Although, a glyphosate-resistant creeping bentgrass (*Agrostis stolonifera* L.) has been developed, the commercial deployment of RR in turfgrasses has yet to occur. Development of turfgrass with transgenically derived herbicide resistance traits to such as glyphosate or glufosinate face steep regulatory concerns before they can be released. In contrast, environmental releases of plants with herbicide resistance obtained by non-transgenic means are not subject to government regulation. Thus, *in vitro* selection for herbicide resistance is an attractive alternative technology to transformation and greatly improves the potential for both domestic and international commercialization of herbicide-resistant turfgrass cultivars.

Seashore paspalum (*Paspalum vaginatum,* Swartz) is a warm-season turfgrass that is adapted to coastal environments. This species is generally adapted to the same regions of the world as bermudagrass (*Cynodon dactylon* (L.) Pers.) and has numerous morphological characteristics that make it desirable as a turfgrass. Interest in the use of seashore paspalum as a turfgrass is largely related to its tolerance to salt and other abiotic stresses (Duncan and Carrow, 2000). Golf course architects recommend seashore paspalum for new courses in tropical or sub-tropical coastal areas where salt or water quality are issues. Many existing golf courses around the world have replaced bermudagrass with paspalum (Raymer et al., 2008).

The main difficulty in replacing bermudagrass with paspalum is bermudagrass re-establishment. Bermudagrass is highly competitive and difficult to eradicate once established.

Invasion by bermudagrass and other weedy grasses can greatly reduce the aesthetic value and quality of paspalum turf. Currently there are no herbicides available that selectively control bermudagrass in seashore paspalum. Development of herbicide-resistant paspalum could provide an effective means of managing bermudagrass in paspalum and allow golf course and sporting venues to transition from bermudagrass to seashore paspalum.

Sethoxydim is a grass-specific herbicide, and resistance in other grass species has been reported as a result of one of two possible single base-pair mutations. The most common mutation is an Ile to Leu substitution caused by an A to T mutation at positioned aa position 1781 of acetyl coenzyme A carboxylase. The objective of this research was to develop, identify, characterize, and evaluate the commercial potential of a seashore paspalum mutant genotype conferring high levels of resistance to sethoxydim and other ACCase herbicides.

MATERIALS AND METHODS

Research was initiated to develop a novel source of resistance to sethoxydim in seashore paspalum using *in vitro* selection and regeneration protocols to select for naturally occurring mutations conferring herbicide resistance (Heckart et al., 2010). A dose response experiment was performed to determine the optimum sethoxydim concentration for selection (data not shown). Callus was induced from immature inflorescences then plated on callus induction medium containing 10 μM sethoxydim for selection. Green plants were regenerated from resistant callus, the Ile to Leu mutation documented, and expression of herbicide resistance confirmed using a series of greenhouse dose response studies. Levels of cross resistance to other ACCase inhibiting herbicides registered for use on turfgrass were determined for the 1781 mutant.

The most vigorous herbicide resistance line SR 31.15 was selected for replicated field trials initiated in 2012 and 2013 under high levels of bermudagrass pressure. Genotypes evaluated included SR31.15 (resistant mutant), SeaSpray (susceptible), and a sethoxydim tolerant line SRSSPRY3 developed in a related project. Each line was evaluated for tolerance to the ACCase herbicides sethoxydim, clethodim, and fenoxyprop at monthly 1X and 3X application rates. Data was collected on bermudagrass control and paspalum injury following the monthly herbicide applications.

A third field experiment was initiated in 2014 with second generation sethoxydim resistant breeding lines obtained through embryo rescue in 2013. Six lines all containing the 1781 mutation conferring ACCase resistance were planted in a similar study to the field studies initiated in 2012 and 2013. Lines tested included the sethoxydim resistant lines SR 31.15-1, -4, -5, -7, -14, and -15; and susceptible controls UGA 1743 and Mauna Key. Herbicides, herbicide rates tested, and data collected were as described above in earlier field experiments.

RESULTS

The effects of sethoxydim rate on injury ratings at 21 days after treatment (DAT) are shown in Fig. 1. The newly developed SR 11 mutant line with the 1781 mutation expressed high levels of resistance (less than 6% injury) to sethoxydim even at rates as high as 3200 grams a.i. ha^{-1}, a rate 15 times the labeled rate for centipedegrass. In contrast, the parental line, Mauna Kea had injury scores of >50% at rates of 200 g a.i. ha^{-1} or higher.

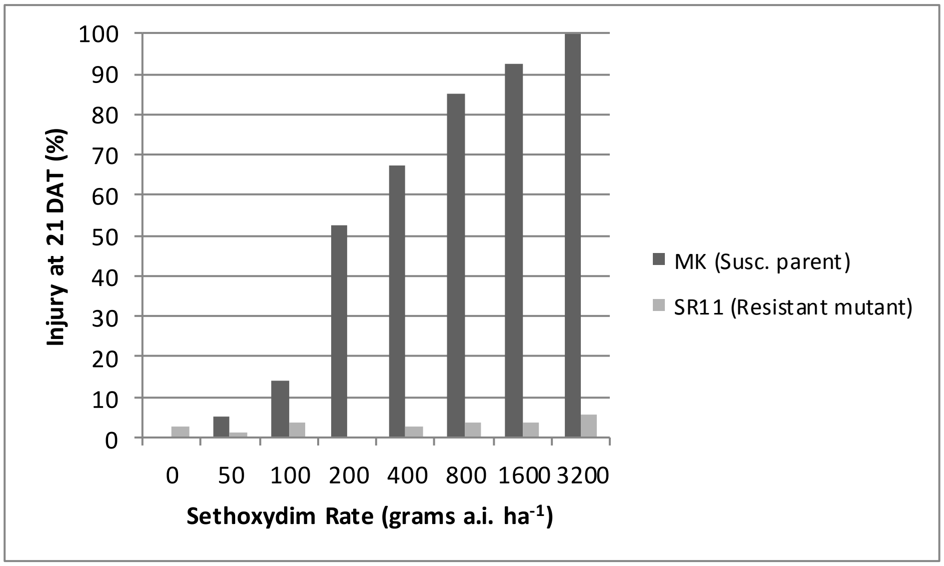

Figure 1. Whole-plant response of resistant SR11 mutant line and MK susceptible parent to a broad range of sethoxydim rates.

Cross resistance dose response studies indicated that the mutant line SR11 showed significantly less injury than the susceptible PT and TCC controls at all fenoxaprop and fluazifop rates above 50 g a.i. ha^{-1} (Table 1). SR11 was also more tolerant to clethodim than the susceptible checks but only at rates up to 200 g a.i. ha^{-1}.

Table 1. Response of parental type (PT), tissue culture control (TCC), and sethoxydim resistant (SR11) to other ACCase inhibiting herbicides.

Herbicide	Plant Injury 21 DAT‡								
	Clethodim (Envoy)			Fenxoxyprop (Acclaim)			Fluazifop (Fusilade)		
Rate†	PT	TCC	SR11	PT	TCC	SR11	PT	TCC	SR11
	%								
0	13.8a§	2.5a	6.2a	8.8a	16.2a	11.2a	12.5b	35.0a	6.2b
50	85.0a	70.0a	22.5a	47.5a	60.0a	10.0b	63.8a	53.8a	10.0b
100	60.0ab	67.0a	42.0b	68.8a	70.0a	8.8b	76.2a	66.2a	12.5b
200	56.3a	52.5a	25.0b	82.5a	91.2a	10.0b	58.8a	70.0a	12.5b
400	85.0a	75.0a	80.0a	92.5a	96.2a	13.8b	83.8a	86.2a	20.0b
800	100.0a	95.8a	100.0a	93.8a	98.8a	12.5b	97.5a	95.0a	50.0b
1600	100.0a	100.0a	100.0a	98.8a	100.0a	8.8b	100.0a	100.0a	40.0b

† Grams a.i. ha^{-1}

‡ DAT = days after treatment.

§ Means on the same row (herbicide rate) and within a herbicide group (i.e. Clethodim) followed by the same letter are not considered to be significantly different at 0.05 according to a protected LSD.

Field studies initiated in 2012 and 2013 indicate that several of the herbicide treatments tested were effective in controlling bermudagrass. Monthly applications of 1X and 3X the recommended rates of fenoxaprop and 3X rates of sethoxydim and clethodim all provided effective control of bermudagrass in test plots. However, the first generation herbicide lines tested lacked vigor and did not perform well enough to be considered for commercial release.

Second generation herbicide resistant lines under evaluation in the 2014 field trial offer renewed promise for development of a non-genetically modified herbicide resistance system for turfgrass.

CONCLUSIONS

A novel form of herbicide resistance in seashore paspalum was developed using tissue culture to select for a naturally occurring mutation known to confer high levels of herbicide resistance to sethoxydim with cross resistance to other ACCase herbicides. This novel germplasm offers great hope for the development of a non-genetically modified herbicide resistance system to provide the ability to manage bermudagrass and other problematic grassy weeds in seashore paspalum.

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