Arkansas Wheat Promotion Board
Annual and Quarterly Report

Title: Hoelon-Resistant Ryegrass in Wheat

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Update: The sixth year of field research is completed but much to be accomplished. Many herbicide treatment options for Hoelon-resistant ryegrass had been tested and we found some good ones. The most promising so far is the new experimental herbicide, Osprey (mesosulfuron) applied postemergence. The best preemergence treatment is Finesse (chlorsulfuron + metsulfuron). The most reliable results come from sequential treatments such as Axiom followed by Everest or Finesse followed by Osprey. Ryegrass from Arkansas are generally annuals (Italian ryegrass). These are morphologically distinct from perennial ryegrass, poison ryegrass, and rigid ryegrass. However, there is great diversity within the Italian ryegrass species that impacts their relative competitive abilities with wheat. Forty-seven ryegrass accessions from Arkansas were DNA-fingerprinted. Genetic data showed that ryegrass from Arkansas generally fall into five genetic clusters. Cases of resistance to Hoelon increased mostly because of independent mutation events triggered by herbicide selection pressure. Movement of seed from resistant ryegrass plants also contributed to increased occurrence of resistant populations. We have identified genetic mutations in the resistant plants and are in the process of developing genetic markers that will expedite the confirmation of resistance.

Objective 1: To develop an Integrated Weed Management system for Hoelon-resistant ryegrass in wheat (Oliver, Scott, and Barrentine).

To evaluate alternative herbicide programs for control of Hoelon (diclofop)-resistant Italian ryegrass (*Lolium multiflorum*), experiments were conducted at Willow Beach, Pine Tree, and Fayetteville. The most outstanding resistant ryegrass control during the past 5 years has been the new experimental herbicide Osprey (mesosulfuron) which should be labeled within the next year. Our testing has shown Osprey controls 96 to 100% of 3-leaf to 2-tiller ryegrass. However, this year under extreme ryegrass pressure (30/ft^2) and cold spraying conditions at Fayetteville Osprey applied at 4 leaf to 2 tiller ryegrass did not provide effective control (78%) and wheat yield was reduced. Sequential postemergence applications of Axiom (flufenacet + metribuzin) fb (followed by) Everest (flucarbazone) provided excellent (90%) control. Finesse (chlorsulfuron + metsulfuron) was the most effective preemergence treatment. A preemergence treatment Finesse fb Osprey also gave equivalent control to the best treatments. Thus, most consistent control programs involved sequential treatments. The important concept to obtain wheat yield equivalent to the weed-free check has been maintaining 80% ryegrass control for the first 5 months following emergence.

Osprey postemergence requires an adjuvant such as a surfactant or crop oil concentrate. Our testing has shown that AgriDex (1%) was as effective as Destiny (0.75 qt/A) or Hasten (0.75 qt/A) plus UAN-28% (2 pt/A). Osprey does not control bulbous buttercup (*Ranunculus bulbosos*) or pineappleweed (*Matricaria matricaroides*). Tank-mixes with Harmony Extra (thifensulfuron and tribenuron), Peak (prosulfuron), Rave (triasulfuron and dicamba), or Amber (triasulfuron) did control ryegrass and the broadleaf weeds mentioned.

A Clearfield wheat cultivar production system was evaluated at Willow Beach and Fayetteville. In the past, Beyond (imazamox) has been best when applied at 1- to 2- leaf wheat or with a repeat application at 3- to 4-leaf wheat. However, this year under extreme ryegrass pressure (22/ft^2) and cold temperature around spray application at Fayetteville, a single application of Beyond at 1-leaf wheat to 3-tiller resistant ryegrass was not effective. The treatments provided initial control but only for 2 to 3 months and wheat yields were reduced 50% compared to sequential treatments. Other excellent treatments were Axiom fb Beyond, Beyond fb Osprey, Sencor (metribuzin) fb Sencor, and Sencor fb Beyond. Finesse applied preemergence also maintains effective season-long control. The imidazolinone herbicide Beyond has proven to be another valuable option for control of resistant ryegrass in wheat.

Objective 2: To conduct week biology studies on Hoelon-resistant ryegrass (Oliver).

Resistance in *Lolium sp.* is one of the most economically important examples of herbicide resistance in world agriculture, and diclofop-resistant ryegrass is the number one weed problem in Arkansas wheat. A field experiment was conducted in 2001 and 2002 at the

Agricultural Experiment Station, Fayetteville, to determine morphological differences among *Lolium sp.* and Arkansas diclofop-resistant ryegrass populations. Ryegrass seeds were collected from Craighead, Crittenden, Cross, Desha, Faulkner, Independence, Lawrence, Lee, Lonoke, Monroe, Perry, Poinsett, Prairie, Randolph, St. Francis, White, and Woodruff counties in Arkansas, and Dunkin, Pemiscot, and Stoddard counties in Missouri. Ryegrass populations from New Jersey, Mississippi, and Oregon and five populations from overseas (Turkey, Uruguay, Netherlands, Soviet Union, and Australia) were included in the study. The experimental design was a randomized complete block with ten replications. During the growing season, plant height, plant growth habit, plant color, and node color were recorded. At maturity, plant height and number of tillers and spikes were recorded. Two spikes from each plant were collected to measure spike and spikelet length, distance between spikelets, awn length, number of spikelets/spike, number of seed/spikelet, number of seed/spike, and total seed/plant.

In general, three growth characteristics were noted: erect, prostrate, and moderately prostrate or spreading ascending. Three ryegrass color types were noted: reddish for prostrate and moderately prostrate; less reddish (red at node and at base of the plant) for erect and moderately prostrate; and greenish for erect and prostrate. Node color was red or green. The populations from Arkansas, Missouri, Mississippi, Uruguay, and the Netherlands were identified as Italian ryegrass. New Jersey and Oregon populations were identified as perennial ryegrass (*Lolium perenne*), populations from Soviet Union and Australia were identified as rigid ryegrass (*Lolium rigidum)*, and the population from Turkey was identified as poison ryegrass (*Lolium temulentum).* At the seedling stage poison ryegrass can be distinguished by a larger diameter main stem (2 to 3 times) and droopy leaves with a wider leaf blade. At maturity, poison ryegrass had the lowest number of tillers (58), spikes (38), seed/spikelet (6), and seed/plant (4,224); fat seed with long awns (12 mm); and glumes longer than spikelet (21 mm) compared to the others. Perennial ryegrass was also easy to distinguish due to prostrate growth habit, a greenish node, short and narrow leaf blade, small seed (<5 mm) with no awn, and glume shorter than spikelet (11 mm). Perennial ryegrass had 188 tillers, 100 spikes, and 18,000 seed/plant and flowered 3 weeks later than the other species.

All of the resistant-ryegrass populations from Arkansas were identified as Italian ryegrass which had erect to prostrate growth habit, greenish to reddish in color, green to red node, glume shorter than spikelet (10 to 14 mm), and medium seed size (>5 mm) with 1- to 3-

mm awns. Within Arkansas, the Italian ryegrass from Woodruff county accession had the highest number of tillers (287). Plants from St. Francis county had the highest number of spikes (226) spikes. Number of tillers and spikes from Independence county populations did not differ from those of Woodruff and St. Francis, but Independence county populations had the highest number of tillers (262), spikes (210), spikelet/spike (25), seed/spikelet (13), and seed/plant (71,234). White county populations had the lowest number of tillers (136), spikes (126), spikelet/spike (22), seed/spikelet (10), and seed/plant (28,063). Therefore, morphological variability exists in Arkansas Hoelon-resistant Italian ryegrass.

Objective 3: Genetic analysis of resistant ryegrass populations (Burgos and Talbert).

In 2003, we confirmed two more cases of ryegrass resistance to Hoelon. Both samples came from St. Francis county. It is apparent that Hoelon-resistant ryegrass is still a problem in wheat production.

Hoelon kills ryegrass by binding to the acetyl coenzyme-A carboxylase (ACCase) enzyme. Continuous use of Hoelon, or several other herbicides, can select for individuals with genetic mutations in the binding site, which result in plant resistance to the herbicide. Gene sequences of ACCase from resistant and susceptible ryegrass were compared and genetic mutations were identified. A partial DNA fragment, encoding the carboxytransferase (CT) domain of the chloroplast ACCase, from several Hoelon-resistant annual ryegrass plants was sequenced. For comparison, the CT region of susceptible individuals was also sequenced. Primers were synthesized based on highly conserved regions of the ACCase amino acid sequence of wheat and corn to partially amplify the CT region. Four sets of overlapping fragments encoding partial ryegrass ACCase was generated. These fragments coded for a total of 573 amino acids. Some neutral mutations in the ACCase nucleotide sequence were observed between resistant and susceptible ryegrass biotypes; however, the resistant plants do not contain an isoleucine (Ile)-1,781 to leucine (Leu) substitution. A change from Ile to Leu in this position of the CT domain was previously shown to confer resistance to grass killers belonging to cyclohexanedione ('dims') family. The mutation in these populations lies downstream in the CT region, as is the case recently reported in blackgrass (Ile-2,041 to Asn).

Based on the gene sequences published recently for blackgrass, we designed two gene-specific, nested primer pairs to clone a sequence of 1082 base pairs. This fragment encompasses the entire CT domain of the ACCase gene. In the initial gene amplification runs we included four individuals from one resistant population of annual ryegrass. Sequences obtained from the four individuals revealed six point mutations resulting in an alteration of the amino acid sequence and these mutations were consistent among all the individuals. The point mutations along with the change in amino acid sequences are listed below.

Nucleotide position	Nucleotide mutation	Altered amino acid
112	AGU→GGU	Ser→Gly
137	AAA→AGA	Lys→ Arg
382	AUG→UUG	Met→Leu
469	AUU→GUU	Ile→Val
509	AUA→ACA	Ile→Thr
971	UCU→UUU	Ser→Phe

We focused our attention on the mutation at nucleotide position 469 that results in a change of Ile to Val, because this mutation has been implicated in Hoelon resistance in rigid ryegrass. This particular mutation results in the introduction of a restriction site, which enables us to cut the CT domain at this location, using the enzyme *Xmn*1. Thus far, we were able to discriminate the resistant plants from susceptible ones using *Xmn*1. In addition, point mutation at nucleotide position 971 introduces a site that can be cut by enzymes *Mnl*1 and *Eco*RV. We are currently analyzing the possibility of using these enzymes to produce molecular markers associated with resistant individuals. In this case, the assay for resistance becomes quick and simple. For mutations that do not result in the introduction of a restriction site, we will utilize gene specific dCAPS (derived cleaved amplified primer sequences) to differentiate the mutated from the normal allele. We are presently in the process of sequencing the CT region of plants from two other resistant populations.

Forty-seven accessions of ryegrass from Arkansas were DNA-fingerprinted. Analysis of genetic data revealed that the ryegrass accessions generally fall into five genetic clusters. This is not surprising because of the morphological diversity that we observed among

Italian ryegrass accessions from within the state. Some genetic diversity is also expected because ryegrass species, except for poison ryegrass, are obligate outcrossers. Based on the distribution of accessions into different clusters, we concluded that resistance to Hoelon in ryegrass has spread around the state mostly because of independent mutations triggered by herbicide selection pressure. There are also cases wherein resistant populations were spread by seed.

Publications

Bararpour, M.T, L.R. Oliver, and N.R. Burgos. 2002. Vegetative and reproductive characteristics of Arkansas resistant ryegrass. Abstr. Res. Conf. Arkansas Crop Prot. Assoc. 6:12-13.

Bararpour, M.T., L.R. Oliver, N.R. Burgos. 2003. Morphological characteristics of the Arkansas diclofop-resistant ryegrass population. Proc. South. Weed Sci. Soc. 56:187.