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Identification and Biological Characteristics of Ryegrass (*Lolium* spp.) Accessions in Arkansas

Mohammad T. Bararpour, Jason K. Norsworthy, Nilda R. Burgos, Nicholas E. Korres, and Edward E. Gbur*

Italian ryegrass is a major weed problem in wheat production worldwide. Field studies were conducted at Fayetteville, AR, to assess morphological characteristics of ryegrass accessions from Arkansas and differences among other *Lolium* spp.: Italian, rigid, poison, and perennial ryegrass. Plant height, plant growth habit, plant stem color, and node color were recorded every 2 wk until maturity. The number of tillers per plant, spikes per plant, and seeds per plant were recorded at maturity. All ryegrass accessions from Arkansas were identified as Italian ryegrass, which had erect to prostrate growth habit, green to red stem color, green to red nodes, glume (10 mm) shorter than spikelet (19 mm), and medium seed size (5 to 7 mm) with 1 to 3 mm awns. However, significant variability in morphological characteristics was found among Arkansas ryegrass accessions. When *Lolium* species at the seedling stage (1- to 2-wk-old plants) were compared, poison ryegrass was characterized as having a large main-stem diameter and wide droopy leaves, whereas perennial ryegrass exhibited a short and a very narrow leaf blade. These two can be distinguished from Italian and rigid ryegrass, which have leaf blades wider than perennial ryegrass but narrower than poison ryegrass. Italian and rigid ryegrass are difficult to distinguish at the seedling stage but are distinct at the reproductive stage. At maturity, Italian ryegrass and poison ryegrass seeds are awned, but perennial and rigid ryegrass seeds are awnless. Poison ryegrass awns were at least 4-fold longer than Italian ryegrass awns. Perennial ryegrass flowered 3 wk later than the other species. Poison ryegrass glumes were longer than the spikelets, whereas Italian ryegrass glumes were shorter than the spikelets. Morphological traits indicate that some Italian ryegrass populations are potentially more competitive and more fecund than others.

Nomenclature: Italian ryegrass, *Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot.; perennial ryegrass, *Lolium perenne* L.; poison ryegrass *Lolium temulentum* L.; rigid ryegrass, *Lolium rigidum* Gaudin; wheat, *Triticum aestivum* L.

Key words: Comparative morphology; discriminant analysis; morphological characteristics; weed identification; weed management.

Ryegrass species are native to the Mediterranean region but are now widely distributed throughout temperate areas of the world (Styles 1986). The genus *Lolium* of the Gramineae family includes eight ryegrass species: Italian, perennial, rigid, Dalmatian (*L. subulatum* Vis.), Canary Islands (*L. canariense* Steud.), poison, hardy (*L. remotum* Schrank.), and Persian darnel (*L. persicum* Boiss. & Hohen ex Boiss.). The general biosystematics of *Lolium*, reviewed by Terrell (1968), may be briefly summarized as follows: (1) all taxa for which chromosome numbers have been counted are diploid with $2n = 14$); (2) perennial, Italian, and perhaps rigid are self-incompatible and cross-pollinated; (3) poison, hardy, and perhaps *L. rigidum* var. *rottbollioides* are self-compatible and self-pollinated. According to Terrell (1968), there are two compatible groups in *Lolium*, the self-pollinated poison and hardy and the cross-pollinated perennial and Italian. *Lolium* spp. are among the most troublesome grass weeds, mainly because of a high capacity to evolve complex herbicide resistance patterns (Campagna et al. 2001; Heap 2016; Kotoula-Syka et al. 2000; Powles and Matthews 1996; Salas et al. 2013).

Herbicide-resistant biotypes of weeds are an increasing threat to crop production. An understanding of the basic biology of resistant biotypes, including their growth and development relative to susceptible biotypes, may yield information helpful in the management of resistance (Crooks et al. 2005). A consequence of herbicide resistance in weed biotypes may be reduced "fitness" (i.e., a less "fit" biotype produces fewer progeny) compared

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with susceptible biotypes (Gressel and Segel 1982). Many reports compare vegetative growth characteristics (e.g., competitiveness, biomass, height, leaf area) of resistant and susceptible biotypes rather than fecundity (Watkinson and White 1985). Although vegetative characteristics are often highly correlated with reproductive parameters, they are not synonymous. The magnitude of differences in vegetative growth and competitiveness, if any, varies with herbicide family and plant species. Herbicide resistance, however, may not have a detectable effect on growth characteristics. For some species, canopy height, biomass, and seed yield were similar for weed biotypes with resistance or susceptibility to cyclohexanedione, dinitroaniline, and organoarsenical and sulfonylurea herbicides (Harris et al. 1995; Holt and Thill 1994; Park et al. 2004; Wiederholt and Stoltenberg 1996). Moreover, weed biotypes resistant to acetolactate synthase (ALS)-inhibiting herbicides may exhibit similar vegetative growth characteristics and competitiveness relative to susceptible biotypes of the same species (Alcocer-Ruthling et al. 1992; Christoffoleti et al. 1997; Lamego et al. 2011; Marshall et al. 2001; Massinga et al. 2005; Park et al. 2004; Thompson et al. 1994). Nevertheless, even in cases in which susceptible and resistant ryegrass biotypes are physiologically similar, they may present different characteristics regarding their ability to compete with other cultures. The more adapted biotypes are usually more competitive and are therefore capable of increasing their dominance with time and eliminating the biotypes that are less fit to occupy a certain ecological niche (Oliveira et al. 2014).

Among herbicide-resistant weeds, rigid ryegrass is perhaps the most infamous. Rigid ryegrass is the first example of a weed that demonstrated multiple resistance to many herbicide families, including aryloxyphenoxyproprionates, cyclohexanediones, sulfonylureas, and dinitroanilines (Powles et al. 1990). The patterns of resistance are variable, reflecting the genetic diversity of the species and the management practices imposed (Heap and Knight 1986; Powles et al. 1997). Resistance in rigid ryegrass is one of the most economically important examples of herbicide resistance in world agriculture (Powles et al. 1997).

Italian ryegrass has been listed as the top weed with resistance to 15 sites of action in the United States (Heap 2016) and is one of the 10 most troublesome weeds of wheat in 10 of the 13 southern U.S. states (Webster and Nichols 2012). Italian ryegrass populations as low as 10 plants m^{-2} reduced wheat yield by 4% (Liebl and Worsham 1987). At 93 plants m^{-2}, wheat yield was reduced by 61% (Appleby et al. 1976). Diclofop-methyl has been used for selective control of Italian ryegrass in wheat in the United States since the early 1980s. Italian ryegrass, which has evolved resistance to diclofop, is the number one weed problem in Arkansas wheat (Barapour 2007, 2012) and is also a serious and widespread weed problem, mostly in grain fields, in many other states across the United States (Heap 2016). The majority of Italian ryegrass populations in the southern United States are now resistant to diclofop; 25% are cross-resistant to pinoxaden (Salas et al. 2013). Most diclofop-resistant populations (about 80%) are also resistant to ALS inhibitors, showing complex resistance patterns to mesosulfuron, imazamox, and pyroxsulam (Salas et al. 2013). The latest problem is increasing resistance to glyphosate (Dickson et al. 2011).

Weed identification at the seedling stage is key to selecting the most effective herbicide control measure, as the response of weed species differs within the same genus (Burgos et al. 2011; Mathis and Oliver 1980; McClelland et al. 1978). Therefore, it is necessary to study the vegetative and reproductive characteristics of ryegrass for the correct identification of ryegrass species. The aim of this research was to evaluate which morphological characteristics of ryegrass accessions from Arkansas can be used for proper species identification. The objectives were to (1) identify common vegetative and phenological characteristics of ryegrass collected from various Arkansas counties; (2) compare a representative type of these accessions with known poison, rigid, and perennial ryegrass specimens; and (3) suggest a simplified identification key that can be used for effective ryegrass management.

Materials and Methods

Sampling and Experimental Layout. Ryegrass seeds were collected from Arkansas, Craighead, Crittenden, Cross, Desha, Faulkner, Independence, Lawrence, Lee, Lonoke, Monroe, Perry, Poinsett, Prairie, Randolph, St. Francis, White, and Woodruff counties in Arkansas wheat fields prior to crop harvest. The rigid, poison, and perennial ryegrass seeds were obtained from Western Regional Plant Introduction Station, Pullman, WA 99164.

Seeds were sown in pots filled with Canadian sphagnum peat moss and wood pulp (SunGro Horticulture, Agawam, MA) on October 18, 2001, and November 5, 2002, for the first and second

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year, respectively, under greenhouse conditions (30/20 C day/night temperature and 14 h photoperiod). On November 12, 2001, and November 22, 2002, 2- to 3-leaf ryegrass seedlings were transplanted in the field, 1.5 by 1.5 m apart. Field studies were conducted at the University of Arkansas Agricultural Experiment Station, Fayetteville, AR (36.099221° N, 94.179184° W), on a Pembroke silt loam (fine-silty, mixed, mesic Mollic Paleudalfs) with 30% sand, 52% silt, 18% clay, 1% organic matter, and a pH of 6.6.

The experimental design was a randomized complete block design with 10 replications, each consisting of all ryegrass accessions collected across the state. The experimental site was sprayed with glyphosate at 0.84 kg ae ha^{-1} prior to ryegrass seedling transplant to eliminate existing vegetation. All transplanted ryegrass seedlings were covered with paper cups (with holes cut on top) for 4 wk to protect the transplants from freeze injury.

Data Collection. During periods of active growth (April to June), qualitative (plant growth habit, plant stem color, and node color) and quantitative (plant height) traits were recorded every 2 wk. At maturity, final plant height and numbers of tillers and spikes were recorded. Two spikes per plant per replication were collected to measure spike and spikelet length, awn length, number of spikelets per spike, number of seeds per spikelet, number of seeds per spike, and number of seeds per plant [(number of seeds per spikelet) × (number of spikelets per spike) × (number of spikes per plant)]. All weeds were removed within a 1 m radius of each ryegrass plant every other week. The rest of the field was mowed with a rotary mower. The field was not irrigated due to timely rainfall throughout the spring months.

Statistical Analysis. Analysis of variance (ANOVA) using PROC GLM in SAS 9.3 for Windows was conducted for data analysis. Because the qualitative morphological characteristics of ryegrass accessions and species were the same both years, data were analyzed with years as a random variable. A hierarchical cluster analysis was performed on height to determine the ryegrass groups based on height; this was then used as one taxonomic identifier of species. Based on pseudo *F*-statistic and cubic clustering criteria, three clusters were chosen.

Discriminant analysis was performed to differentiate the variation between ryegrass species, which were assigned as categorical variables, and their reproductive characteristics, which were assigned as covariates. The means over all observations were obtained and then standardized across accessions to ensure that the analysis was not influenced by the units of measurement of the variables. The linear function was then used, assuming that the covariance matrices within each species are equal while the covariate means differ between groups.

Results and Discussion

Characteristics of Ryegrass Accessions. Arkansas ryegrass accessions exhibit distinguishable differences based on both vegetative (recorded during the vegetative period) and reproductive (recorded during the reproductive period) characteristics. These are described in the following sections.

Vegetative Characteristics. Arkansas ryegrass accessions can be classified in three groups in terms of plant height. These include tall (77 to 82 cm), intermediate (74 to 76 cm), and short (68 to 73 cm) plants (Table 1; Figure 1). The accessions from Lawrence, Lonoke (AR-99-28), Monroe, Poinsett, and St. Francis counties were all classified as tall, whereas those from Arkansas, Crittenden, Desha, Faulkner, Lee, Lonoke (AR-99-33), Perry, and Prairie counties were short. Accessions from Craighead, Cross, Faulkner, Independence, Lonoke (AR-98-16), Poinsett, Randolph, St. Francis, White, and Woodruff counties were classified as intermediate (Table 1). At harvest, the ryegrass accession from Lawrence County was the tallest (82 cm), whereas the accession from Prairie County was the shortest (69 cm). The differences in height between ryegrass accessions reflect differential competitive ability within these accessions (Fraga et al. 2013). The taller accession is expected to dominate the competitive relationship through shading (Blair 2001) if its competitor, wheat for example, is shorter. Bararpour et al. (2012) reported that the natural infestation of Italian ryegrass in Arkansas reduced wheat yield an average of 75%.

Ryegrass plants from Lawrence and Lee counties had a prostrate growth habit, and plants from Arkansas, Poinsett, White, and Woodruff counties had spreading– ascending (SA) growth characteristics (Table 1). Accessions from Cross, Faulkner (AR-97-10), and Monroe counties had a combination of erect (60%) and SA plants (40%).

All other ryegrass accessions, except those collected from Faulkner (AR-98-10), Independence,

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Table 1. Vegetative characteristics of Italian ryegrass accessions from Arkansas during the growing season.[a]

Accession code[a]	County	Growth habit[b]	Stem color[b]	Node color[b]	Final height (cm)
AR-97-10	Faulkner	E(SA)[b]	G	Re	76
AR-98-1	St. Francis	E	G	Re	78
AR-98-4	Craighead	E	G	Re	73
AR-98-8	Faulkner	E	G	Re	73
AR-98-10	Faulkner	P(SA, E)	G	Re	73
AR-98-16	Lonoke	E	G	Re	75
AR-98-18	Lee	P	G	Re(G)	72
AR-99-9	Desha	E	G	Re	73
AR-99-14	Prairie	P(SA)	G	G	69
AR-99-17	Monroe	E(SA)	G	G(Re)*	77
AR-99-20	Arkansas	SA	G	Re	71
AR-99-28	Lonoke	E	G	Re	79
AR-99-33	Lonoke	SA(P)	G	Re	72
AR-99-36	Perry	SA(E)	G(Re)	Re	73
AR-99-40	Lawrence	P	G(Re)	Re	82
AR-99-43	Crittenden	E	G	Re	71
AR-99-44	Poinsett	SA	G	Re	78
AR-99-47	White	SA	G(Re)	Re	75
AR-99-48	Randolph	E	G	Re	74
AR-99-52	Independence	E(P)	G(Re)	Re	75
AR-99-54	Woodruff	SA	G	Re	74
AR-99-56	Cross	E(SA)	G	Re	76
AR-99-61	St. Francis	E	G	Re	75
LSD(0.05)					5

[a] Abbreviations: AR, Arkansas; SA, spreading–ascending; E, erect; P, prostrate; G, green; Re, red.
[b] Letters in parentheses indicate variants in the population. An asterisk (*) indicates a recessive characteristic with regard to color.

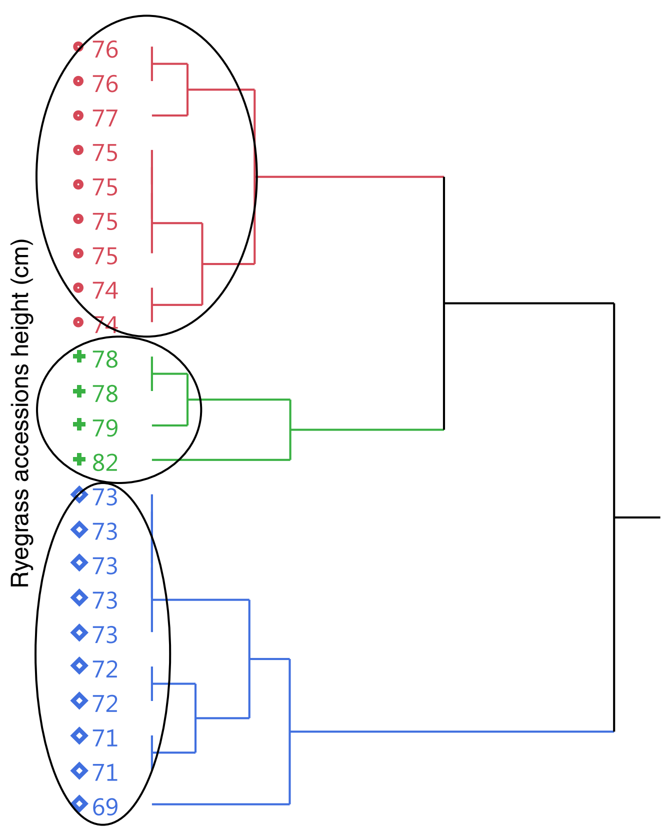

Figure 1. Ryegrass height classification based on hierarchical clustering

Lonoke (AR-99-33), Perry, and Prairie, exhibited an erect growth habit. An erect growth habit could provide a competitive advantage to a weed against a crop, as it enhances capture and use of resources, particularly photosynthetic active radiation, by the weed (Korres and Froud-Williams 2002).

Stem color in most accessions was dark green, except for ryegrass from Independence, Lawrence, Perry, and White counties, which included plants (40%) with red pigmentation (Table 1). Most ryegrass plants had red nodes, except those from Prairie County, which had green nodes; ryegrass plants from Lee and Monroe counties had either green or red nodes in proportion 60:40 for each county.

The number of tillers of accessions from Lawrence and Craighead counties did not differ from those of Crittenden, Cross, Desha, Faulkner (AR-98-8), Lonoke (AR-99-28 and AR-99-33), Monroe, Perry, Poinsett, Prairie, and Woodruff (Table 2). The ryegrass accession from White County had the lowest number of tillers (108 plant^{-1}) compared with those with 181 tillers plant^{-1} (AR-98-4 and AR-99-40). Increases in biomass production or its components, including tillers, are positively related to increased competitiveness for both crop plants and weeds, as in the

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Table 2. Vegetative and reproductive characteristics of Italian ryegrass accessions from Arkansas.

Accession code[a]	County	Tillers plant^{-1}	Spikes plant^{-1}	Spikelets spike^{-1}	Seed spikelet^{-1}	Seed plant^{-1}
AR-97-10	Faulkner	141	120	22	10	30,386
AR-98-1	St. Francis	179	146	23	10	36,746
AR-98-4	Craighead	181	129	23	9	29,741
AR-98-8	Faulkner	152	124	24	10	34,171
AR-98-10	Faulkner	141	120	22	10	30,386
AR-98-16	Lonoke	138	110	23	10	28,661
AR-98-18	Lee	142	120	24	10	31,991
AR-99-9	Desha	149	135	24	11	41,697
AR-99-14	Prairie	166	123	21	9	26,249
AR-99-17	Monroe	148	131	23	11	36,671
AR-99-20	Arkansas	134	118	22	11	30,249
AR-99-28	Lonoke	161	130	25	11	36,393
AR-99-33	Lonoke	169	145	23	10	37,169
AR-99-36	Perry	154	133	23	10	34,321
AR-99-40	Lawrence	181	153	22	11	38,373
AR-99-43	Crittenden	164	133	24	11	37,950
AR-99-44	Poinsett	164	144	23	10	38,990
AR-99-47	White	108	100	22	9	20,524
AR-99-48	Randolph	144	124	22	9	24,569
AR-99-52	Independence	173	145	24	12	44,909
AR-99-54	Woodruff	180	133	25	10	36,822
AR-99-56	Cross	152	118	23	10	31,776
AR-99-61	St. Francis	142	115	22	9	25,273
LSD (0.05)		36	32	2	1	10,395

[a] Abbreviation: AR, Arkansas

case of cereals, particularly winter wheat, and blackgrass (*Alopecurus myosuroides* Huds.) (Chauvel et al. 2002). The number of spikes (reproductive structure of ryegrass) increased with the number of tillers, which would ultimately increase the ryegrass soil seedbank. Lemerle et al. (1979) reported reductions in crop tillering and inflorescence formations resulting in poor grain yield due to Italian ryegrass interference. Alshallash and Drennan (1993) recorded substantial decreases in both total dry weight and grain yield relative to the weed-free crop when Italian ryegrass was growing with a range of wheat densities (0, 25, 50, 100, and 200 plants m^{-2}) with and without 100 weed plants m^{-2}. Hashem et al. (1998) measured reductions up to 92% in winter wheat yield due to competition from Italian ryegrass.

Reproductive Characteristics. Ryegrass from Lawrence County had the greatest number of spikes (153 spikes plant^{-1}), while ryegrass from White County had the lowest number (100 spikes plant^{-1}) (Table 2). The number of spikes per plant of the accession from Lawrence County did not differ from those of Craighead, Crittenden, Desha, Faulkner (AR-98-8), Independence, Lonoke (AR-99-28 and AR-99-33), Monroe, Perry, Poinsett, Prairie, Randolph, St. Francis (AR-98-1), and Woodruff counties. Accessions from Arkansas, Cross, Faulkner (AR-97-10, AR-98-8, and AR-98-10), Lee, Lonoke (AR-98-16 and AR-99-28), Randolph, Prairie, St. Francis (AR-99-61), and White counties had similar numbers of spikes.

Ryegrass from Independence County had 24 spikelets spike^{-1}, while ryegrass from Prairie County had 21 spikelets spike^{-1} (Table 2). The number of spikelets per spike from Independence County did not differ from that of Lawrence, and the number of spikelets per spike from Prairie County did not differ from White County. Ryegrass had 9 to 12 seeds spikelet^{-1} (Table 2). The Independence County accession, with 12 seeds spikelet^{-1}, and the accessions from Craighead, Prairie, Randolph, St. Francis (AR-99-61), and White counties, with 9 seeds spikelet^{-1}, had the highest and lowest number of seeds per spikelet, respectively. However, the number of seeds per spikelet from the Independence County accession did not differ from those of Arkansas, Crittenden, Desha, Lawrence, Lonoke (AR-99-28), and Monroe counties; and the number of seeds per spikelet from the White County accession also did not differ from those of

Craighead, Cross, Faulkner, Lee, Lonoke (AR-98-16 and AR-99-33), Perry, Poinsett, Prairie, Randolph, St. Francis, and Woodruff counties. Tillers plant^{-1} were highly related with spikes plant^{-1} and seeds plant^{-1} (r^2=0.82 and 0.62, respectively) (Figures 2A and 2B). In addition spikes plant^{-1} were highly related with seed plant^{-1} (r^2=0.82) (Figure 2C). The high correlation between tillers per plant, spikes per plant with seeds per plant highlights the importance of these variables in the reproductive capacity of ryegrass and their usefulness as classification tools. These variables convey information about the potential contribution of ryegrass species or populations to soil seedbanks if not controlled effectively.

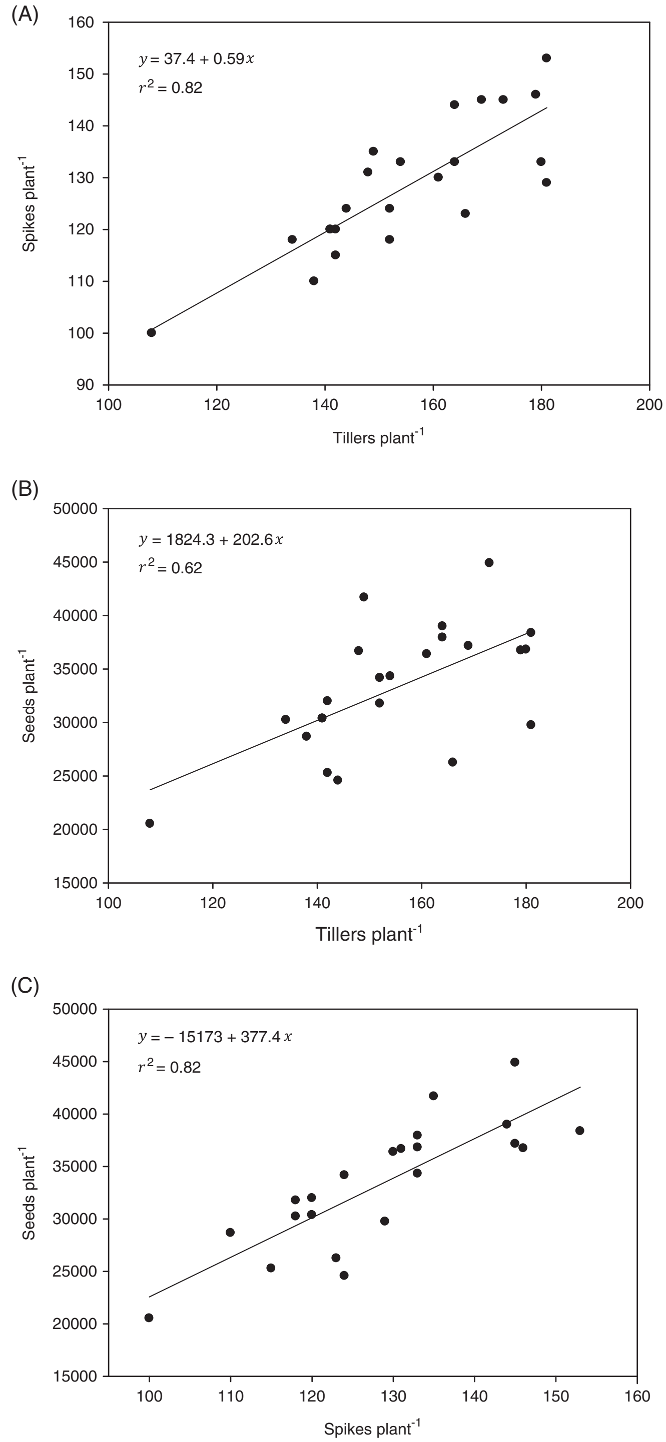

Figure 2. Comparisons of reproductive characteristics among Arkansas ryegrass accessions grown under field conditions and correlation of (A) number of tillers and spikes production; (B) number of tillers and seed production; and (C) number of spikes and seed production.

The Monroe County accession had the longest spike (29 cm) and the Faulkner accession (AR-98-10) had the shortest (24 cm) (Table 3); spikelet lengths ranged from 1.5 (Prairie) to 2 cm (Lawrence) (Table 3). The Monroe County accession had the longest glume (13 mm) and Woodruff County had the shortest (10.2 mm). All ryegrass accessions from Arkansas County were awned. The ryegrass from Arkansas, Independence, Lawrence, and Randolph counties had the longest awns (3.6 and 4.1 mm), whereas those from Monroe and Poinsett counties had the shortest awns (1.9 mm). Ryegrass accessions from the state of Arkansas had an erect to prostrate growth habit, dark green stems or green stems with red pigmentation, green to red nodes, glumes shorter than spikelets, and medium seed size (5 to 7 mm) with 1.9 to 4 mm awns. The accession from Lawrence County had the highest number of spikes, and ryegrass from White County had the lowest number of spikes. In terms of seeds per spikelet, ryegrass from Independence County had 12 seeds spikelet^{-1} and that from White County had 9 seeds spikelet^{-1}. The number of spikelets per spike differed among ryegrass accessions. Spike, spikelet, glume, and awn lengths also differed among ryegrass accessions.

Evidence in this research and in accordance with existing literature (Yatskievych 1999) indicates that the accessions collected from Arkansas counties belong to Italian ryegrass, a highly competitive species (Alshallash and Drennan 1993; Hashem et al. 1998; Lemerle et al. 1979). Competitiveness pertains to the ability of an organism (weed species in this case) to perform better in acquiring resources in relation to another organism (crop plants) within the same habitat (Korres et al. 2016). Weediness, which comprises traits that secure the survival and dispersal of weeds, even under severe environmental conditions, can be described through various morphological, phenological, or physiological characteristics (Korres et al. 2016). The high genetic diversity among weedy plants allows them to achieve

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Table 3. Selected reproductive characteristics of Italian ryegrass accessions from Arkansas.

Accession code[a]	County	Spike (cm)	Spikelet (cm)	Glume (mm)	Awn (mm)
AR-97-10	Faulkner	27	1.8	11.1	3.2
AR-98-1	St. Francis	26	1.7	11.5	2.1
AR-98-4	Craighead	26	1.6	12.2	2.0
AR-98-8	Faulkner	27	1.8	12.0	3.2
AR-98-10	Faulkner	24	1.7	11.0	2.0
AR-98-16	Lonoke	26	1.7	11.7	2.4
AR-98-18	Lee	27	1.7	11.0	3.0
AR-99-9	Desha	26	1.7	11.6	2.5
AR-99-14	Prairie	25	1.5	11.3	3.4
AR-99-17	Monroe	29	1.9	13.0	1.9
AR-99-20	Arkansas	25	1.8	11.6	4.1
AR-99-28	Lonoke	28	1.9	12.4	3.0
AR-99-33	Lonoke	26	1.7	11.1	2.4
AR-99-36	Perry	26	1.8	10.8	2.9
AR-99-40	Lawrence	28	2.0	11.6	3.6
AR-99-43	Crittenden	26	1.8	12.1	3.3
AR-99-44	Poinsett	27	1.7	11.4	1.9
AR-99-47	White	26	1.6	11.0	3.1
AR-99-48	Randolph	25	1.7	10.9	3.6
AR-99-52	Independence	26	1.9	10.4	3.6
AR-99-54	Woodruff	26	1.8	10.2	3.2
AR-99-56	Cross	25	1.6	11.0	2.5
AR-99-61	St. Francis	25	1.7	10.8	2.1
LSD (0.05)		2	NS	1.3	1.2

[a] Abbreviation: AR, Arkansas

a greater competitive fitness against crops under diverse environmental conditions (Dukes and Mooney 1999). Reproductive capacity is linked to resource capture (DeFelice et al. 1988; Benvenuti and Steffani 1994), which is related to increased biomass and leaf area (Korres 2005).

Morphological Differences among a Representative Arkansas Ryegrass Accession of Rigid, Poison, and Perennial Ryegrass. A representative Arkansas ryegrass accession (AR-99-28 from Lonoke County) was compared with known rigid, poison, and perennial ryegrass species. Italian ryegrass (AR--99-28) from Lonoke County was chosen as a representative species because of its resemblance to the general population of Arkansas ryegrasses (visual observations of wheat fields around the state of Arkansas) in terms of tiller number, seed production, color, and growth habit characteristics. Significant differences either in vegetative or reproductive characteristics were found across species.

Vegetative Characteristics. At 2 wk after emergence, poison ryegrass had a larger main stem (2- to 3-fold) compared with the other species. Poison ryegrass also had droopy leaves. Perennial ryegrass had the narrowest leaf blade (2 to 3 mm) among ryegrass species. In general, three leaf-blade size categories were classified. Poison ryegrass had the widest leaf blade, 10 to 11 mm; Italian and rigid ryegrass had similar leaf widths of 5 to 6 mm. At the seedling stage (2 wk old), ryegrass has either a narrow or wide leaf blade. If the leaf blade is narrow (2 mm width), it is perennial ryegrass. Ryegrass with a wide leaf blade is either poison, Italian, or rigid ryegrass. If the ryegrass leaf blade is 10 to 11 mm wide, it is poison ryegrass. But if the ryegrass leaf blade is 5 to 6 mm in width, it is either Italian or rigid ryegrass. It is difficult to distinguish between Italian and rigid ryegrass at the 2-wk-old seedling stage. However, poison and perennial ryegrass are easy to distinguish from one another or from Italian or rigid ryegrass. Growth habit can also be used as a rough guide to distinguish ryegrass species, because Italian and poison ryegrass exhibit an erect growth habit, whereas perennial ryegrass is prostrate, and rigid ryegrass is either erect or prostrate (Table 4).

Stem color comparison was based on a representative Italian ryegrass accession (AR-99-28), which had greenish stems. Rigid ryegrass stem color varied between green (60%) and reddish (40%), whereas poison and perennial ryegrass had the same color as Italian ryegrass. Poison and perennial ryegrass had green nodes, but Italian and rigid ryegrass had red nodes. Apparently, stem and node color cannot reliably differentiate Italian from rigid ryegrass.

Table 4. Vegetative characteristics of Italian, rigid, perennial, and poison ryegrass.[a]

Ryegrass species	Accession code	Growth characteristics[b]	Stem color[b]	Node color	Final height (cm)
Italian	AR-99-28	E	G	Re	79
Rigid	323257	E (P)	G(Re)	Re	44
Perennial	L143-8-Pinn	P	G	G	29
Poison	545646	E	G	G	76
LSD (0.05)					7

[a] Abbreviations: AR, Arkansas; L, Lolium; E, erect; P, prostrate; G, green; Re, red
[b] Letters in parentheses indicate variants in the population.

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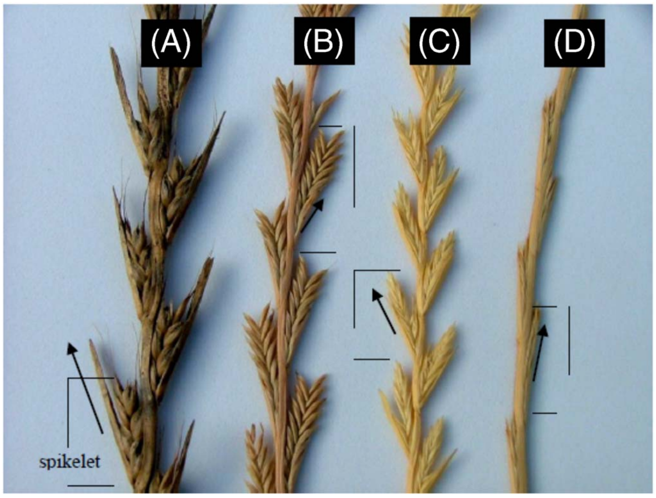

Figure 3. Spikes of four ryegrass species: (A) poison ryegrass, (B) Italian ryegrass, (C) perennial ryegrass, and (D) rigid ryegrass. Arrow indicates glume length and box indicates spikelet length.

Growth habit is not a reliable key to distinguish Italian and rigid ryegrass. Italian ryegrass, being 79 cm tall and with the highest number of tillers, may be the most competitive of these species (Table 4; Figure 3). This is the species that primarily infests wheat fields in Arkansas. Italian and poison ryegrass had similar heights (Table 4). Rigid ryegrass was taller than perennial ryegrass. Italian ryegrass had more tillers than the other ryegrass species (Figure 3). An alternative way to distinguish Italian from rigid ryegrass is by reproductive characteristics.

Reproductive Characteristics. Italian, poison, and rigid ryegrass flowered at the same time (late April), while perennial ryegrass flowered mid- to late May. At maturity, Italian ryegrass and poison ryegrass seed had awns, but perennial and rigid ryegrass seed did not (Table 5). The awn length of poison ryegrass was at least 4-fold longer than that of Italian ryegrass. The key traits that distinguish ryegrass species at the reproductive stage, particularly Italian and rigid species, are the presence or absence of awns, spikelets, and glumes. Ryegrass seed is either awned or awnless. If ryegrass seed is awnless, it is perennial or rigid ryegrass. If the seed is awned, it is either Italian or poison ryegrass. Poison ryegrass has an awn greater than 10 mm long, but Italian ryegrass has an awn shorter than 5 mm. The other key characteristic to distinguish poison ryegrass from either Italian, rigid, or perennial ryegrass is that the glume is longer than the spikelet in poison ryegrass, while the glume is shorter than the spikelet in Italian, rigid, and perennial ryegrass.

Table 5. Comparison of reproductive characteristics of four ryegrass species.

Ryegrass species	Accession code[a]	Spike (cm)	Spikelet (mm)	Glume (mm)	Awn (mm)
Italian	AR-99-28	28	19	12	3
Rigid	323257	17	15	12	0
Perennial	L143-8-Pinn	19	13	11	0
Poison	545646	25	17	23	13
LSD (0.05)		2	1	2	1

[a] Abbreviation: AR, Arkansas; L, Lolium

Italian and poison ryegrass have longer spikes and spikelets than either rigid or perennial ryegrass. The glume of poison ryegrass was almost twice the length of any other ryegrass species (Table 5; Figure 4). The poison ryegrass glume (23 mm) was longer than the spikelet (17 mm), whereas the Italian ryegrass glume (12 mm) was shorter than the spikelet (19 mm). Poison ryegrass awns (>10 mm) were at least two times longer than Italian ryegrass awns (<5 mm).

The glume was shorter than the spikelet in Italian, rigid, and perennial ryegrass, but the glume was longer than the spikelet in poison ryegrass (Table 5; Figure 4). In rigid and perennial ryegrass, the glume was longer than two-thirds of the spikelet length, but the glume was shorter than two-thirds of the spikelet in Italian ryegrass.

Italian ryegrass had the highest number of spikes per plant, and poison ryegrass had the lowest number of spikes per plant. Rigid and perennial ryegrass had the same number of spikes per plant (Figure 3). Italian ryegrass had the highest number of spikelets per spike, and rigid ryegrass had the lowest number of spikelets per spike (Figure 3). Poison and perennial ryegrass had the same number

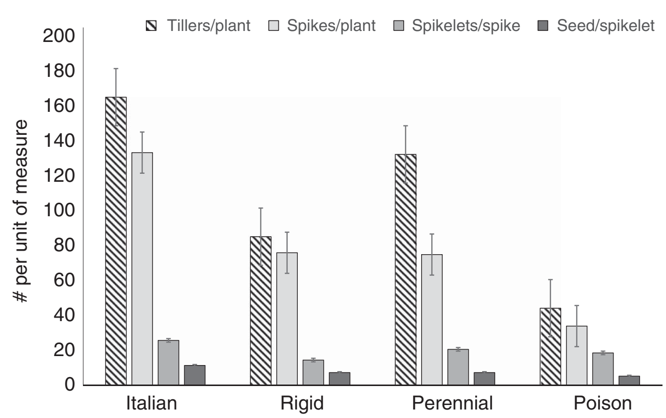

Figure 4. Comparisons of reproductive characteristics (tillers per plant, spikes per plant, spikelets per spike, and seeds per spikelet) among Italian, rigid, perennial, and poison ryegrass grown under field conditions.

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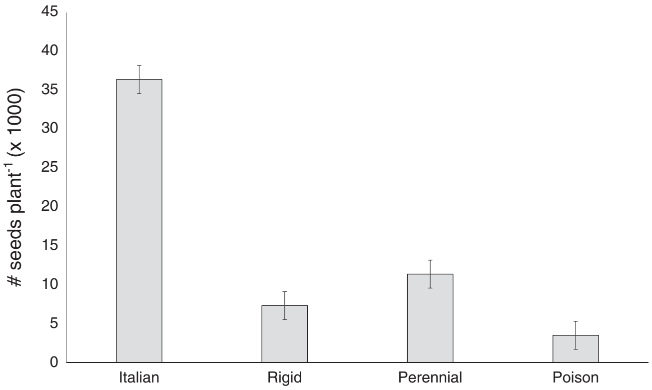

Figure 5. Seed production capability of Italian, rigid, perennial, and poison ryegrass.

of spikelets per spike. Italian ryegrass had the highest number of seeds per spikelet (11 to 12), and poison ryegrass had the lowest number of seeds per spikelet (5 to 6). Rigid and perennial ryegrass had the same number of seeds per spikelet (Figure 3). Italian ryegrass has longer spikes and spikelets than rigid, poison, or perennial ryegrass. Italian ryegrass, being the tallest among ryegrass species and with the highest number of tillers, produced the highest number of spikes per plant (Table 4; Figure 3).

High number of tillers and seeds per spikelet in Italian ryegrass resulted in the highest number of seeds at 36,393 seed plant^{-1}. Poison ryegrass, having the lowest number of tillers and seeds per spikelet, produced the lowest number of seeds at 3,501 seed plant^{-1} (Figure 5). The highest number of tillers and spikes per plant in Italian ryegrass resulted in the highest number of seeds per plant compared with the other ryegrass species tested in this experiment. Italian ryegrass produced 3.2, 5.0, and 10.4 times the number of seeds per plant compared with perennial, rigid, and poison ryegrass, respectively (Figure 5).

Overall, in Arkansas, Italian ryegrass was capable of producing up to 180 tillers and up to 45,000 seed plant^{-1}.

The capacity of Italian ryegrass to produce high numbers of seeds per plant and subsequently increase seed deposition to the soil seedbank is a major problem not only in Italian ryegrass control measures but also in evolution of herbicide-resistant Italian ryegrass. The results presented in this work indicate the importance of Italian ryegrass in Arkansas wheat fields. As Bararpour and Oliver (2007) reported, the natural infestation of Arkansas Italian ryegrass (±323 plants m^{-2}) interference reduced wheat yield an average of 72% over 6 yr.

It is important to be able to distinguish Italian, rigid, poison, and perennial ryegrass at vegetative and reproductive stages for effective ryegrass management practices. Differences in susceptibility of the individual species indicated the importance of proper species identification before herbicide application (Mathis and Oliver 1980; McClelland et al. 1978). These morphological and reproductive characteristics will enable producers to identify Italian ryegrass, which is the main problematic ryegrass species in wheat fields in the mid-southern United States, and to distinguish Italian from rigid, perennial, or poison ryegrass. If the correct identification of ryegrass species has been made then a case of herbicide failure is most likely due to a resistant biotype rather than misidentification. Although ryegrass accessions from Arkansas were identified as Italian ryegrass, the existing morphological variability reflects the genetic diversity of ryegrass (Heap and Kight 1986; Powles et al. 1990), which is influenced by the prevailing environmental conditions.

Based on discriminant analysis of vegetative traits, Italian ryegrass accessions from Arkansas did not clearly separate into distinct groups (nondistinct morphological differences) despite observable differences (Figure 6). Therefore, Italian ryegrass in Arkansas belongs to one vegetative morphotype. Discriminant analysis of vegetative and reproductive traits revealed that the ryegrass accessions formed distinct groups by species, and despite the morphological diversity within the Italian ryegrass species, it is still taxonomically distinct from the other ryegrass species.

Keys for Ryegrass Identification. Classification for ryegrass recognition at vegetative and reproductive stages based on the results presented in this

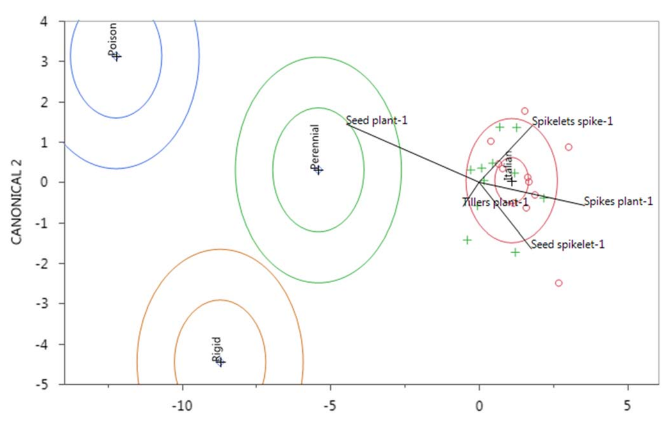

Figure 6. Ryegrass species classification on vegetative and reproductive characteristics as determined by discriminant analysis.

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work is summarized below. This classification system is user-friendly, as it combines data from the detailed analysis of both qualitative and quantitative ryegrass characteristics.

Vegetative Key

- If leaf blade is narrow (2 mm), then it is perennial ryegrass
- If leaf blade is wide (5 to 10 mm), then it is
  - 5 to 6 mm = Italian and rigid ryegrass else
  - 10 to 11 mm = poison ryegrass

Reproductive Key

- If awn is absent, then it is perennial or rigid ryegrass
- If awn is present, then it is Italian or poison ryegrass, and then
  - glume $<$ spikelet and awn $<$ 5 mm = Italian ryegrass, else
  - glume $>$ spikelet and awn $>$ 10 mm = poison ryegrass

Literature Cited

Alcocer-Ruthling M, Thill DC, Shafii B (1992) Differential competitiveness of sulfonylurea resistant and susceptible prickly lettuce (*Lactuca serriola*). Weed Technol 6:303–309

Alshallash KS, Drennan DSH (1993) Competition between spring wheat and *Lolium multiflorum* (Italian ryegrass). Proceedings of the Brighton Crop Protection Conference, Brighton, UK, November 13–16, 1993. Weeds 1:113–117

Appleby AP, Olson PD, Colbert DR (1976) Winter wheat yield reduction from interference by Italian ryegrass. Agron J 68:463–466

Bararpour MT, Oliver LR (2007) Comparison of wheat herbicides for control of Arkansas diclofop-resistant Italian ryegrass. Ark Crop Prot Assoc 11:1

Bararpour MT, Oliver LR, Norsworthy JK (2012) Herbicide screening for Arkansas diclofop-resistant Italian ryegrass control in wheat. Page 105 *in* Proceedings of the 2012 Southern Weed Science Society, Volume 65, Weed Management in Agronomic Crops. Charleston, SC: SWSS

Benvenuti M, Steffani A (1994) Effects of shade on reproduction and some morphological characteristics of *Abuthilon theophrasti*, *Datura stramonium* L. and *Sorghum halepense* L. Pers. Weed Res 34:283–288

Blair B (2001) Effect of soil nutrient heterogeneity on the symmetry of belowground competition. Plant Ecol 156: 199–203

Burgos NR, Shivrain VK, Scott RC, Mauromoustakos A, Kuk YI, Sales MA, Bullington J (2011) Differential tolerance of weedy red rice (*Oryza sativa* L.) from Arkansas, USA to glyphosate. Crop Prot 30:986–994

Campagna C, Gasparetto MA, Sattin M (2001) Situation and management of grass weeds resistant to ACCase inhibitors in Italy. Pages 755–762 *in* Proceedings of the Brighton Conference on Weeds. Farnham, UK: BCPC Registered Office

Chauvel B, Munier-Jolain NM, Grandgirard D, Gueritaine G (2002) Effect of vernalization on the development and growth of *Alopecurus myosuroides*. Weed Res 42:166–175

Christoffoleti PJ, Westra P, Moore F (1997) Growth analysis of sulfonylurea-resistant and susceptible kochia (*Kochia scoparia*). Weed Sci 45:691–695

Crooks HL, Burton MG, York AC, Brownie C (2005) Vegetative growth and competitiveness of common cocklebur resistant and susceptible to acetolactate synthase-inhibiting herbicides. J Cotton Sci 9:229–237

DeFelice MS, Witt WW, Barrett M (1988) Velvetleaf (*Abutilon theophrasti*) growth and development in conventional and no-till corn (*Zea mays*). Weed Sci 36:609–615

Dickson JW, Scott RC, Burgos NR, Salas RA, Smith KL (2011) Confirmation of glyphosate-resistant Italian ryegrass (*Lolium perenne* ssp. *multiflorum*) in Arkansas. Weed Technol 25: 674–679

Dukes JS, Mooney HA (1999) Does global change increase the success of biological invaders? Trends Ecol Evol 14:135–139

Fraga DS, Agostinetto D, Vargas L, Nohatto MA, Thurmer L, Holz MT (2013) Adaptive value of ryegrass biotypes with low-level resistance and susceptible to the herbicide fluazifop and competitive ability with the wheat culture. Planta Daninha, Viçosa-MG 31:875–885

Gressel J, Segel LA (1982) Interrelating factors controlling the rate of appearance of resistance: the outlook for the future. Pages 325–348 *in* LeBaron HM & Gressel J, eds. Herbicide Resistance in Plants. New York: Wiley

Harris JR, Gossett BJ, Toler JE (1995) Growth characteristics of selected dinitroaniline-resistant and susceptible goosegrass (*Eleusine indica*) populations. Weed Technol 9:562–567

Hashem A, Radosevich SR, Roush ML (1998) Effect of proximity factors on competition between winter wheat (Triticum aestivum) and Italian ryegrass (*Lolium multiflorum*). Weed Sci 46:181–190

Heap I (2016) The International Survey of Herbicide Resistant Weeds. www.weedscience.org. Accessed: January 21, 2016

Heap J, Knight R (1986) The occurrence of herbicide cross-resistance in a population of annual ryegrass *Lolium rigidum* resistant to diclofop-methyl. Aust J Agric Res 37:149–156

Holt JS, Thill DC (1994) Growth and productivity of resistant plants. Pages 299–316 in Powles SB & Holtrum JAM, eds. Herbicide Resistance in Plants: Biology and Biochemistry. Boca Raton, FL: Lewis

Korres NE (2005) Encyclopaedic Dictionary of Weed Science: Theory and Digest. Paris: Intercept and Lavoisier. 695 p

Korres NE, Froud-Williams RJ (2002) Effects of winter wheat cultivars and seed rate on the biological characteristics of naturally occurring weed flora. Weed Res 42:417–428

Korres NE, Norsworthy JK, Tehranchian P, Gitsopoulos TK, Loka DA, Oosterhuis DM, Gealy DR, Moss SR, Burgos NR, Miller MR, Palhano M (2016) Cultivars to face climate change effects on crops and weeds: a review. Agron Sustain Dev 36:1–22

Kotoula-Syka E, Tal A, Rubin B (2000) Diclofop-resistant *Lolium rigidum* from northern Greece with cross-resistance to ACCase inhibitors and multiple resistance to chlorsulfuron. Pest Manag Sci 56:1–5

Lamego FP, Vidal RA, Burgos NR (2011) Competitiveness of ALS inhibitors resistant and susceptible biotypes of greater beggarticks (*Bidens subalternans*). Planta Daninha, Viçosa-MG 29:457–464

Lemerle D, Michael PW, Sutton BG (1979) The competitive abilities of wheat and triticale against different densities of *Lolium rigidum*. Pages 447–450 *in* Proceedings of 7th Asian-Pacific Weed Science Society Conference. Sydney, Australia, November 26–30, 1979

Liebl RA, Worsham AD (1987) Interference of Italian ryegrass (*Lolium multiflorum*) in wheat (*Triticum aestivum*). Weed Sci 35:819–823

Marshall MW, Al-Khatib K, Loughlin T (2001) Gene flow, growth, and competitiveness of imazethapyr-resistant common sunflower. Weed Sci 49:14–21

Massinga RA, Al-Khatib K, Amand PS, Miller JF (2005) Relative fitness of imazamox-resistant common sunflower and prairie sunflower. Weed Sci 53:166–174

Mathis WD, Oliver LR (1980) Control of six morningglory (*Ipomoea*) species in soybeans (*Glycine max*). Weed Sci 28: 409–415

McClelland MR, Oliver LR, Mathis WD, Frans RE (1978) Responses of six morningglory (*Ipomoea*) species to bentazon. Weed Sci 26:459–464

Oliveira C, Agostinetto D, Vargas L, Avila LA, Tarouco CP (2014) Does resistance to glyphosate herbicide affect the competitive ability of ryegrass with soybean? Planta Daninha, Vicosa-MG 32:189–196

Park KW, Mallory-Smith CA, Ball DA, Mueller-Warrant GW (2004) Ecological fitness of acetolactate synthase inhibitor-resistant and -susceptible downy brome (*Bromus tectorum*) biotypes. Weed Sci 52:768–773

Powles SB, Holtum JAM, Matthews JM, Liljegren DR (1990) Herbicide cross-resistance in rigid ryegrass (*Lolium rigidum* Gaud.). Pages 394–406 *in* Managing Resistance to Agrochemicals. American Chemical Society Symposium Series 421. Washington, DC: American Chemical Society

Powles SB, Matthews JM (1996) Integrated weed management for the control of herbicide resistant annual ryegrass (*Lolium rigidum*). Pages 407–414 *in* Proceedings of the Second International Weed Control Congress, Copenhagen, Denmark, June 25–28, 1996

Powles SB, Preston C, Bryan IB, Jutsum AR (1997) Herbicide resistance: impact and management. Adv Agron 58:57–93

Salas RA, Burgos NR, Mauromoustakos A, Lassiter RB, Scott RC, Alcober EA (2013) Resistance to ACCase and ALS inhibitors in *Lolium perenne* ssp. multiflorum in the United States. J Crop and Weed 9:168–183

Styles BT (1986) Intraspecific Classification of Wild and Cultivated Plants. Oxford, UK: Clarendon

Terrell E (1968) A Taxonomic Revision of the Genus *Lolium* (Technical Bulletin No. 1392). Washington, DC: Agricultural Research Service, USDA

Thompson CR, Thill DC, Shafii B (1994) Growth and competitiveness of sulfonylurea-resistant and susceptible kochia (*Kochia scoparia*). Weed Sci 42:172–179

Watkinson AR, White J (1985) Some life-history consequences of modular construction in plants. Philos Trans R Soc London, Ser. B 313:31–51

Webster TM, Nichols RL (2012) Changes in the prevalence of weed species in the major agronomic crops of the Southern United States: 1994/1995 to 2008/2009. Weed Sci 60: 145–157

Wiederholt RJ, Stoltenberg DE (1996) Absence of differential fitness between giant foxtail (*Setaria faberi*) accessions resistant and susceptible to acetyl-coenzyme A carboxylase inhibitors. Weed Sci 44:18–24

Yatskievych G (1999) 76. Lolium L. (ryegrass). Pages 841–845 *in* Flora of Missouri, Volume 1. Jefferson City, MO: Department of Conservation, in cooperation with the Missouri Botanical Garden Press, St. Louis, MO

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