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Characterization of Echinochloa spp. in Arkansas

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Crop, Soil and Environmental Sciences

by

Hussain Tahir Universiti Putra Malaysia Bachelor of Science in Bioindustry, 2007

August 2016 University of Arkansas

This thesis is approved for recommendation to the Graduate Council

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Dr. Tom Barber Committee Member Dr. Krishna N. Reddy Ex-Officio Member

OVERALL ABSTRACT

Echinochloa species are the most problematic grass weeds in rice and soybean production throughout the southern United States. The species under this genus has high intra- and interspecific variability, with many ecotypes, observed within a species. This study was conducted to: i) characterize the morphology and phenology of *Echinochloa* accessions from Arkansas in a common garden; ii) verify the species identity of these accessions based on their morphological traits, and iii) evaluate the dormancy characteristics of *Echinochloa* accessions. Junglerice (E. colona) was identified as the most common species, comprising about 78% of the accessions collected, with barnyardgrass (E. crus-galli) and rough barnyardgrass (E. muricata) representing about 9% and 12% of the population, respectively. Thus, junglerice is the most problematic Echinochloa species affecting farmers in Arkansas. The height of junglerice ranged from 65-94 cm; barnyardgrass, 87-126 cm; and rough barnyardgrass, 79-118 cm. Rough barnyardgrass was the largest species with the longest (19.8-42 cm) and widest (1.2-2.1 cm) leaves, followed by barnyardgrass. Junglerice and barnyardgrass were the earliest to flower, at about 39-59 days after planting (DAP), followed by rough barnyardgrass (46-63 DAP). Junglerice produced the largest amount of seed (9,098-217,217 per plant) with an average of 539 seeds per panicle. Barnyardgrass produced 7,186-71,494 seed/plant with an average of 345 seeds per panicle. The dominance of junglerice over the other species could be due to its high seed production and high seed germination capacity. The growth habit of junglerice could be either prostrate, decumbent or open whereas that of barnyardgrass and rough barnyardgrass could be decumbent to open. Junglerice and barnyardgrass had similar panicle characteristics with an almost similar color range that could lead to misidentification. Junglerice had the highest

germination capacity (41-99%), followed by barnyardgrass at 11-79%. Rough barnyardgrass exhibited deep dormancy, with germination capacity between 2 and 73%. Further investigations are needed to characterize the germination requirements of rough barnyardgrass and its relative abundance in North America. The differential tolerance of these species to various herbicides are being investigated in follow-up research. This information is useful in making weed management decisions and is informative in understanding speciation and adaptation of weedy *Echinochloa* species.

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I would like to acknowledge my late Father Mr. Tahir Bin Yacoob who gave me strong support until I was able to do my degree here in the United States of America. To my mother Rashida Packeer Meera Sahib, I am here today because of you. Your love and tender care have brought me until I became a capable man. I would also like to acknowledge Fatimah Zahara, Ibrahim, Abdul Rahman and Abdul Rahim for giving their strong support and taking care of our mom. May God bless all of you. Most importantly, I thank God for the good life and every blessing he has given me.

DEDICATION

This Master's thesis and all work related to this thesis is specially dedicated to My beloved daddy Mr. Tahir Bin Yacoob who taught me the best knowledge is to find it through hardship and My mother, Mrs. Rashida Packeer Meera Sahib.

Special thanks to my sister Fatimah Zahara and my brothers Ibrahim, Abdul Rahman and Abdul Rahim. Thank you for being there for me.

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CHAPTER I INTRODUCTION

INTRODUCTION

Echinochloa is a cosmopolitan genus, with several weedy species, commonly found in most crop production systems especially rice fields. This genus belongs to the Gramineae family (Poaceae), infesting 36 crops in 61 countries, and is well adapted to tropical and warm temperate regions of the world (Holm et al. 1977; Martinez et al. 1999). It occurs between 50° N and 40° S latitude (Maun and Barrett 1986). Some species of this genus are considered the most troublesome weed in crops worldwide. The *Echinochloa* genus has about 50 species occurring in diverse environments (Tabacchi et al. 2006), 19 of which are reported in the United States (USDA NRCS 2014). Four species are reported in Arkansas, including rough barnyardgrass (*Echinochloa muricata*), barnyardgrass (*Echinochloa crus-galli*), junglerice (*Echinochloa colona*) and coast cockspurgrass (*Echinochloa walteri*) (Gentry et al. 2013; Smith 1988).

Early emergence of *Echinochloa* in the rice field results in intense competition with rice. Competition with *Echinochloa* species, especially *E. colona* and *E. crus-galli*, occurs from 15 days after rice emergence to maturity, with yield reduction up to 80% (Smith Jr. 1983). In general, *Echinochloa* species can produce up to 1 million seed per plant (Mitich 1990; Martinkova 1989). *E. crus-galli var. oryzicola*, specifically, can produce up to 40, 000 seed/plant; its seedling looks very similar to rice and, therefore, is usually transplanted with rice unintentionally (Holm et al. 1977). The growth habit of *Echinochloa* species is strongly affected by environmental conditions (Yamasue 1997). Morphological variation in the genus is also significantly influenced by soil texture and fertility level; on many occasions, these local adaptations resulted in the evolution of different ecotypes (Martinez et al. 1999). Barrett (1982) showed distinct variations among *Echinochloa* species accessions sampled more than 10 km apart. *Echinochloa* species collected from different habitats showed significant differences in

height, growth habit, number of tillers, and other traits even though they were from the same locality (Yamasue, 1997). The notoriety of this genus as an economically important weed is fuelled by high seed production, seed dispersal mechanism, dormancy, adaptation to a wide range of photoperiods, and genetic diversity that favors the evolution of resistance to herbicides (Martinez et al. 1999; Maun and Barrett, 1986).

In a survey, *Echinochloa* species was ranked as the number one weed in rice in Arkansas and across the southern United States rice belt (Norsworthy et al. 2013: Dowler 1995). *Echinochloa* species can consume up to 80% of available nitrogen in the soil; application of nitrogen fertilizer will increase its competitiveness (Holm et al. 1977). In highly infested fields, *Echinochloa* reduces rice tiller number, height, number of panicles, grain weight and yield per panicle (Holm et al. 1977). Competition with *Echinochloa* can reduce rice tillers by 50%. *Echinochloa* is a C4 plant; therefore, has high efficiency of carbon fixation compared with most C3 crops like rice and soybean (Holm et al. 1977).

This genus is one of the most difficult taxa to classify into species due to intra-species and inter-species variation with the tendency to intergrade. Sparcino et al. (1994) identified 44% of the *Echinochloa* specimen collected based on taxonomic description; in that research 52% of the specimen exhibited mixed characteristics. Traits that were used previously for species characterization such as length of awn were affected by relative humidity and agronomic practices affect the degree of spikelet disarticulation in some taxa (Michael 2003; Yatskievych 1999). Having purple marks on the leaf blade is not a proper identification key for *E. colona* (Bryson and Reddy 2012). Lack of pictorial treatment and limited biological characterization makes this genus a big challenge for the majority of scientists and crop growers.

Resistance to herbicides is one of the most important concerns in agriculture and weed management will become more complicated as multiple resistances continue to evolve (Manalil et al. 2011). Resistance to herbicides among *Echinochloa* is widespread. Resistance in barnyardgrass had been reported on atrazine, azimsulfuron, bispyribac-sodium, butachlor, clomazone, cyanazine, cyhalofop-butyl, fenoxaprop P-ethyl, imazamox, imazethapyr, molinate, nicosulfuron, pendimethalin, penoxsulam, profoxydim, propanil, quinclorac, quizalofop-P-ethyl, and thiobencarb (Heap 2015). In Arkansas, barnyardgrass has evolved resistance to propanil, quinclorac, imidazolinones and clomazone (Heap 2015; Riar et al. 2015). Propanil-resistant barnyardgrass populations were reported also in Texas, Missouri and Louisiana (Heap 2015).

Junglerice [*E. colona*], a specie most similar to barnyardgrass, also presents the same problem with resistance evolution. Junglerice was reported to be resistant to ametryn, atrazine, azimsulfuron, bispyribac-sodium, cyhalofop-butyl, fenoxaprop P-ethyl, fluazifop-P-butyl, glyphosate, haloxyfop-P-methyl, metribuzin, propanil and quinclorac (Heap 2015). In the United States, this specie was reported to have resistance to glyphosate (in California) and multiple resistance to imazethapyr, propanil and quinclorac in Arkansas (Heap 2015). However, the resistance profiles attributed to *E. colona* and *E. crus-galli* are uncertain because of the confusion between these two species. It is very likely that several of what have been called barnyardgrass are-junglerice.

The specific objectives of this study are to: i) characterize the morphology and phenology of *Echinochloa* accessions from Arkansas in a common garden; ii) verify the species identity of these accessions based on their morphological traits; and iii) evaluate the dormancy characteristics of *Echinochloa* species.

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CHAPTER II

LITERATURE REVIEW

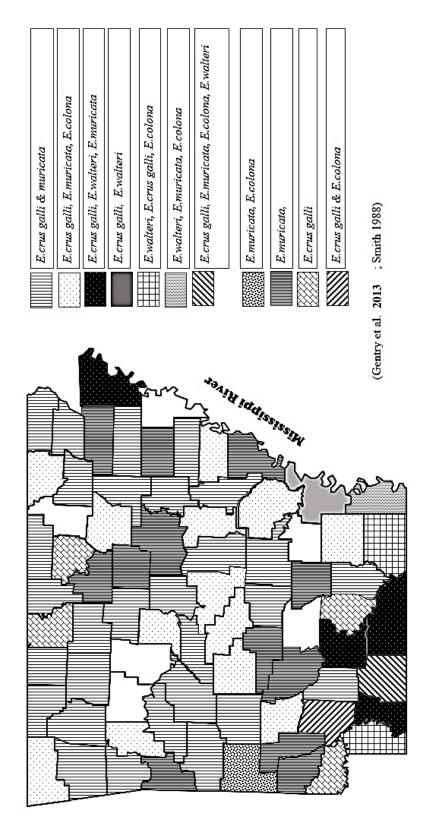
CHAPTER II

LITERATURE REVIEW

Species Diversity of the Echinochloa Genus

There are 19 out of 50 *Echinochloa* species reported in the United States. These species are junglerice (E. colona), barnyardgrass (E. crus-galli), Gulf cockspurgrass (E. crus-pavonis), elliptic cockspurgrass (E. elliptica), Japanese millet (E. esculenta), billion-dollargrass (E. frumentacea), cockspur (E. haploclada), rough barnyardgrass (E. muricata), Chihuahuan cockspur (E. oplismenoides), early watergrass (E. oryzoides), Florida cockspurgrass (E. paludigena), rice barnyardgrass (E. phyllopogon), variegated cockspurgrass (E. picta), creeping rivergrass (E. polystachya), antelopegrass (E. pyramidalis), decorative millet (Echinochloa holciformis), hippograss (E. stagnina), channel millet (E. tumeriana) and coast cockspurgrass (E. walteri) (Tabacchi et al. 2006; USDA NRCS 2014). In Arkansas, there are four species reported, including rough barnyardgrass, barnyardgrass, junglerice and coast cockspurgrass (Gentry et al. 2013). Barnyardgrass and rough barnyardgrass were reported to be widespread in the United States, except Alaska (USDA NRCS 2014). Junglerice was reported in North Carolina, South Carolina, Georgia, Alabama, Tennessee, Mississippi, Arkansas, Louisiana, Oklahoma and Texas (Keener et al. 2016; Gentry et al. 2013; Smith 1988; Bryson and Skojac 2011; Department of Biology Herbarium (TAMU) and Tracy Herbarium 1998; Chester et al. 1993; Thomas and Allen 1993; Duncan 1981; McGregor et al. 1977; Radford et al. 1965). Coast cockspurgrass is present in the majority of states in the United States except California, Oregon, Washington, Montana, Idaho, Nevada, Utah, Arizona, New Mexico, Kansas, Nebraska, South Dakota, North Dakota and Colorado (USDA NRCS 2014).

A study by Bryson and Reddy (2012) of 240 samples collected from Alabama, Arkansas, Kentucky, Louisiana, Mississippi, and Tennessee showed that the most common species in crop production is junglerice followed by rough barnyardgrass, barnyardgrass, and Gulf cockspurgrass (*Echinochloa crus-pavonis* var. *macera*). Coast cockspurgrass was rare in crop fields. They concluded that barnyardgrass and junglerice are the most problematic weeds in rice in the southern United States.





Distribution of Echinochloa in Arkansas

In Arkansas, junglerice was reported in 29 counties including Benton, Carroll, Newton, Izard, Craighead, Poinsett, Mississippi, Crittenden, Lee, Bradley, Drew, Lincoln, Arkansas, Desha, Chicot, Hempstead, Miller, Pike, Prairie, Howard, Independence, Polk, Garland, Pulaski, Pope, Conway, Randolph, Saline and Franklin counties (Figure 2.2) (Gentry et al. 2013; Smith 1988).

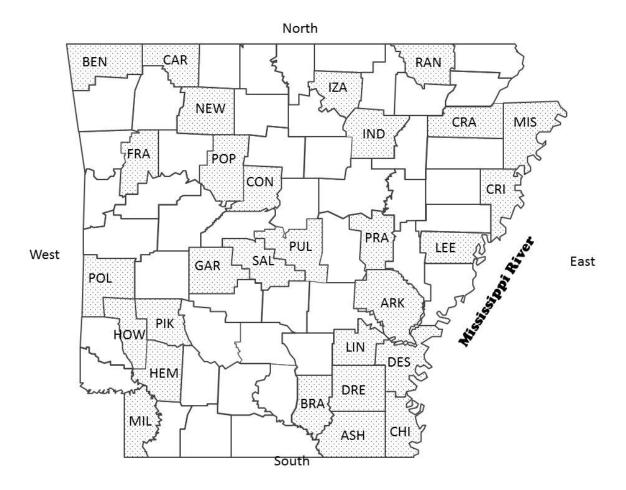


Figure 2.2 Distribution of junglerice in Arkansas, USA. County Code BEN = Benton, CAR = Carroll, NEW = Newton, IZA = Izard, CRA = Craighead, POI = Poinsett, MIS = Mississippi, CRI = Crittenden, LEE = Lee, BRA = Bradley, DRE = Drew, LIN = Lincoln, ARK = Arkansas, DES = Desha, CHI = Chicot, HEM = Hempstead, MIL = Miller, PIK = Pike, PRA = Prairie, HOW = Howard, IND = Independence, POL = Polk, GAR = Garland, Pulaski, POP = Pope, CON = Conway, RAN = Randolph, SAL = Saline, FRA = Franklin (Gentry et al. 2013; Smith 1988)

Barnyardgrass was reported to be widespread in Arkansas except in Johnson, Conway, Stone, Cleburne, White, Cross, Polk, Hot Spring, Howard, Clark, Dallas and Seview counties (Figure 2.3) (Gentry et al. 2013; Smith 1988).

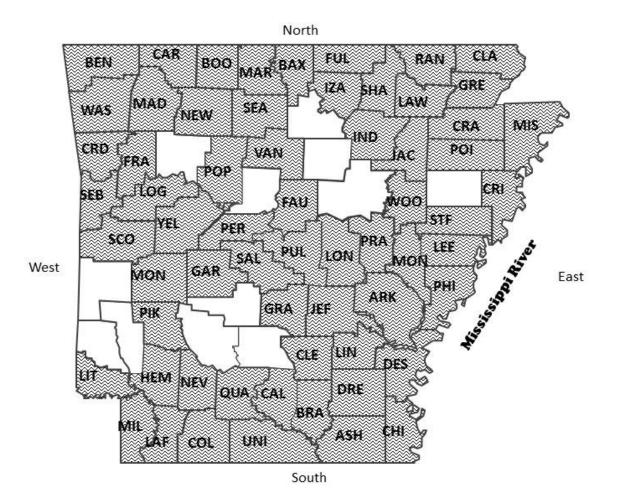


Figure 2.3 Distribution of barnyardgrass in Arkansas, USA. County Code BEN = Benton, CAR =Carroll, BOO = Boone, MAR = Marion, BAX = Baxter, FUL = Fulton, SHA = Sharp, RAN = Randolph, CLA = Clay, GRE = Greene, WAS = Washington, MAD = Madison, NEW = Newton, SEA = Searcy, IZA = Izard, SHA = Sharp, LAW = Lawrence, CRA = Craighead, POI = Poinsett, MIS = Mississippi, CRI = Crittenden, JAC = Jackson, IND = Independence, VAN = Van Buren, POP = Pope, FRA = Franklin, CRD = Crawford, SEB = Sebastian, LOG = Logan, YEL = Yell, PER = Perry, FAU = Faulkner, WOO = Woodruff, STF = St. Francis, LEE = Lee, MON = Monroe, PRA = Prairie, LON = Lonoke, PUL = Pulaski, SAL = Saline, GAR = Garland, MON = Montgomery, PIK = Pike, LIT = Little River, HEM = Hempstead, NEV = Nevada, OUA = Ouachita, CAL = Calhoun, DRE = Drew, GRA = Grant, JEF = Jefferson, LIN = Lincoln, ARK = Arkansas, DES = Desha, CHI = Chicot, MIL = Miller, LAF = Lafayette, COL = Columbia, UNI = Union, ASH = Ashley (Gentry et al. 2013; Smith 1988)

Coast cockspurgrass was reported only in Scott, Ashley, Desha, Chicot, Union,

Columbia, Lafayette, Ouachita, Miller, St. Francis, Crittenden, and Mississippi counties (Figure 2.4) (Gentry et al. 2013; Smith 1988).

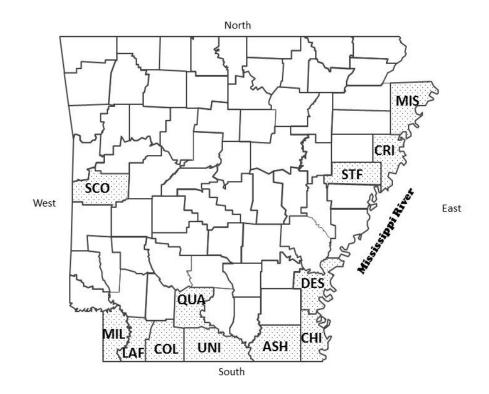
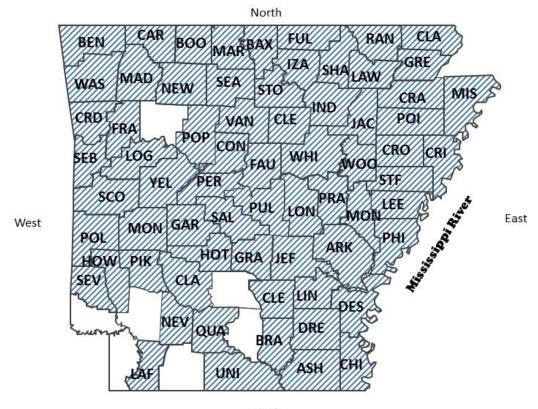


Figure 2.4 Distribution of coast cockspurgrass in Arkansas, USA. County Code MIS = Mississippi, CRI = Crittenden, STF = St. Francis, SCO = Scott, MIL = Miller, LAF = Lafayette, OUA = Ouachita, UNI = Union, ASH = Ashley, CHI = Chicot, DES = Desha (Gentry et al. 2013; Smith 1988)

Rough barnyardgrass was reported in more counties compared with barnyardgrass, being absent only from Johnson, Little River, Miller, Dallas, Hempstead, Calhoun and Columbia counties (Figure 2.5) (Gentry et al. 2013; Smith 1988).



South

Figure 2.5 Distribution of rough barnyardgrass in Arkansas, USA. County Code BEN = Benton, CAR = Carroll, BOO = Boone, MAR = Marion, BAX = Baxter, FUL = Fulton, SHA = Sharp, RAN = Randolph, CLA = Clay, GRE = Greene, WAS = Washington, MAD = Madison, NEW = Newton, SEA = Searcy, IZA = Izard, SHA = Sharp, LAW = Lawrence, CRA = Craighead, POI = Poinsett, MIS = Mississippi, CRI = Crittenden, JAC = Jackson, IND = Independence, VAN = Van Buren, POP = Pope, FRA = Franklin, CRD = Crawford, SEB = Sebastian, LOG = Logan, YEL = Yell, PER = Perry, FAU = Faulkner, WOO = Woodruff, STF = St. Francis, LEE = Lee, MON = Monroe, PRA = Prairie, LON = Lonoke, PUL = Pulaski, SAL = Saline, GAR = Garland, MON = Montgomery, PIK = Pike, NEV = Nevada, OUA = Ouachita, CLK = Clark, HOT = Hot Spring, DRE = Drew, GRA = Grant, JEF = Jefferson, LIN = Lincoln, ARK = Arkansas, DES = Desha, CHI = Chicot, LAF = Lafayette, UNI = Union, ASH = Ashley, CON = Conway, STO = Stone, CLE = Cleburne, WHI = White, POL = Polk, SEV = Sevier, HOW = Howard (Gentry et al. 2013; Smith 1988)

Weediness of Echinochloa species

Several *Echinochloa* species exhibit weedy characteristics such as prolific seed production, prolonged emergence duration due to dormancy, photoperiod insensitivity, and rapid vegetative growth. *Echinochloa* is a C4 plant, which equates to more efficient use of carbon dioxide than C3 plants such as rice and soybean. This characteristic, and various weedy traits, have made *Echinochloa* a great competitor in crop production systems in both flooded (e.g. rice) and non-flooded (e.g. soybean, corn, various others) fields (Bagavathiannan et al. 2011; Mitich 1990). Weedy *Echinochloa* has persisted and defied control measures. Excellent control of *Echinochloa* species will result in increased rice yields. A study by Ottis and Talbert (2007) conducted in Stuttgart, AR, USA between 2002 and 2004 showed that competition with barnyardgrass most affected the total aboveground biomass production, panicle weight, and harvest index of modern rice cultivars (Wells, CL161) or hybrid rice (XL8). Rice yield increased by 750 kg/ha with 10% barnyardgrass control. Season-long competition with an older cultivar, Lemont, reduced rice yield by 70% (Smith 1983). *Echinochloa* species have strong negative impact on the crop, thus, a thorough understanding of the biology of this weed is important.

Taxonomic Information on Echinochloa species

Taxonomic characterization of this genus has been a major challenge to weed scientists (Yatskievych 1999). The confusion about these taxa arises from its continuous morphological and phenological variation, making it difficult to distinguish species within the genus (Barrett and Wilson 1981). Some of the *Echinochloa* species have traits that are intermediate between other species (Michael 2003). The morphological trait limits of these taxa are not well defined, resulting in specimen misidentification (Costea and Tardif 2002; Michael 1983). Limited

availability of photographed treatments also causes problems in identification, which is a major constraint for new scientists studying this genus (Damalas et al. 2008). A few taxonomists have proposed different taxonomic treatments for *Echinochloa* species (Carretero 1981; Costea and Tardif 2002; Gould et al. 1972; Michael 1983; Michael 2001; Pignatti 1982). Characterizations of *Echinochloa* in the Mediterranean region were conducted by Pignatti (1982), which helped in the identification of barnyardgrass in that region. The work of Carretero (1981) helped in the identification of junglerice, barnyardgrass and coast cockspurgrass in Italy, Southern France, Spain, and Portugal. Costea and Tardif (2002) focused on the lemma and caryopsis morphology in their study of five species of *Echinochloa*, including barnyardgrass, thus taking species identification to a complex, microscopic level. Treatments by Yatskievych (1999), Micheal et al. (1994) and Gould et al. (1972) involving the species in North America were used as the main reference for species identification in this region. However, their descriptions also show a wide range, which makes it difficult to distinguish between species based on morphology (Tabacchi et al. 2006). Because of the plasticity of the species, variation in weather conditions and cropping systems also contribute to this problem. *Echinochloa* morphological variation within a species will look different even within the same field. In a study in Italy, only 44.3% (56 out of 129) of the specimen were identified based on the taxonomic key; many plants had intermediate traits and could not be identified with confidence (Sparacino et al. 1994). Information on the biology of other Echinochloa species is limited; most research projects focus on barnyardgrass, without including other species for comparison under the same growing conditions (Damalas et al. 2008).

Description of Echinochloa species

Junglerice (Echinochloa colona)

Importance and habitat: Junglerice is a noxious weed in rice fields with almost the same vegetative morphology as rice (Catindig et al. 2011). It is a C4 tropical specie, which is native to India and an important weed of the main crops grown in more than 60 countries, especially rice (Valverde et al. 2000). It is widespread in the tropics and subtropics between 30°N and 30°S (Michael 1983).

Junglerice grows fast, but does not tolerate drought. In a study by Chauhan and Johnson (2010), the growth and reproduction of junglerice were retarded significantly in water-stressed conditions, specifically showing a reduction in plant height, biomass and seed production (Chauhan and Johnson 2010). In water-stressed conditions, junglerice grew taller than rice, but the germination capacity of seeds from water-stressed plants was not reduced relative to non-stressed plants (Chauhan and Johnson 2010). Because of limited tolerance to flooding, it flourishes in upland or rainfed rice fields (Catindig et al. 2011). It cannot withstand frost and will be killed at temperatures below -9 °C (Ecocrop 2011). This specie will not flourish under shaded environments (FAO 2002a).

Species description. Junglerice is a summer annual grass, about 30 -100 cm tall. It is green to purple in color, tufted and shortly stoloniferous. It has glabrous, cylindrical, erect and decumbent culms. The base of the culm is red purple with roots at the lower node (Catindig et al. 2011; Ecocrop 2011). The leaves are flat, 10-25 cm long, 3-7 mm wide, glabrous, tinged with red at the base and has no ligule. The culm is reddish purple or green, ascending to erect, and rarely has hairs. The inflorescence is green to purple, 6-12 cm long, and normally carries 3-7 racemes on the main axis. The lengths of primary branches are 1.0-2.0 cm, ascending and loosely spaced,

usually not overlapping or only slightly overlapping along the main axis. The spikelet is 2.5-3.0 mm long, awnless, with green to light brown hull when matured. The upper glume is 2.4-3.0 mm long, tapered at the tip to a sharp point. The palea is 2/3-3/4 the size of the lemma and is 2.4-3.0 mm long. The lower lemma is 2.6-2.9 mm long with a soft tip and wrinkled at maturity. The spikelets are arranged in four rows on one side of the racemes (Michael 2003; Yatskievych 1999). This specie flowers throughout the year and propagates by seed. The seeds have a short dormancy period compared with other species in the same genus (Chauhan and Johnson 2009).

Barnyardgrass [Echinochloa crus-galli]

Importance and habitat. Barnyardgrass originated from Europe and India and was reported frequently as the main yield-reducing weed of rice (Smith 1983). It was also reported as the most problematic weed in the temperate and warm environment (Holm et al. 1977). As with junglerice, its seedlings are usually mistaken as rice in rice fields because seedlings look similar to rice. Barnyardgrass infests 36 crops in 61 countries and is considered the most widespread specie in the world (Holm et al. 1991). It grows well in moist areas or wetlands and normally grows in direct-seeded rice fields and wastelands (Catindig et al. 2011).

Species Description. Barnyardgrass is a summer annual that grows erect, tufted or reclining at the base and can grow up to 200 cm tall. The culms root at lower nodes. The bare stem is hairless and filled with spongy white pith. The leaf has a round base and narrow tip. The leaf blade is about 10-40 cm long; 5-30 mm wide, glabrous, and does not have a ligule. The only specie with a ligule under this genus is *Echinochloa polystachya* (Gould et al. 1972). The color of the inflorescence is loose green to purplish. The spikelets are more or less elliptical, pointed, and have hairs on the hull. Inflorescences are 5-25 cm long with the main primary branches 2-8

cm long. Spikelets are 2.8-4.4 mm long (excluding the awns) with green to brown or dark purple hull at maturity. The upper glume is 2.6-4.2 mm long (excluding the awn, if present); the tips are tapered to a sharp point. The lemma is 2.6-4.4 mm long (excluding the awn, if present), the tip is tapered to a sharp point or with an awn 1-40 mm long, and sparsely to densely hairy, at least along the nerves (Yatskievych 1999). This specie can flower throughout the year, and mature in 52-84 days (Vengris et al. 1966).

Due to rapid growth, competitive ability, and high fecundity, barnyardgrass is considered a serious weed in lowland rice. Infestation can be reduced with repeated tillage prior to planting rice to kill emerged cohorts (FAO 2002b).

Coast cockspurgrass (Echinochloa walteri)

Importance and habitat. This specie is a perennial grass that is native to the eastern United States and Canada (Kartesz 1994). This plant grows well in wet environments and is normally found in marshes, swamps, and along the edges of bodies of water, in disturbed or undisturbed sites, but not in rice fields.

Species Description. Coast cockspurgrass is 30-200 cm tall; stout, erect or ascending from often spreading bases; and the culm is about 2.5 cm thick. The blades are up to 55 cm long, 10–35 mm wide, and scabrous. The panicles are 8.5-35 cm long, erect to slightly drooping; with hairs 3.5-5 mm long. The primary branches are 1-10 cm long, ascending and relatively densely spaced, mostly strongly overlapping along the main axis. Secondary branches are present on the longer primary branches. Spikelets are about 3-5 mm long (excluding the awns); usually dark purple at maturity. The upper glume is 2.8-4.8 mm long (excluding the awns), ovate, tapered at the tip to an awn 2-12 mm long, usually roughened or hairy along the nerves. Lower glumes are

usually more than 1/2 as long as the spikelets, abruptly narrowing to a fine, 0.5 mm point; the lower florets are sterile; the lower lemmas are usually awned; awns are 8-25 mm long. The lower palea is subequal to the lower lemmas. The upper lemmas are 3 - 5 mm long, about 1.5 mm wide, not exceeding or scarcely exceeding the upper glumes, narrowly ovate to elliptical, the coriaceous portion is sub-acute, tips are acuminate, membranous, without a line of hairs at the base of the tip. The caryopses are 1.2-1.8 mm long and brownish (Michael 2003; Yatskievych 1999). An occasional specimen of *E. walteri* has glabrous lower sheaths and short awns, which can be distinguished from *E. crus-pavonis* by their less dense panicles (Yatskievych 1999).

Rough barnyardgrass [Echinochloa muricata]

Importance and habitat. This is an annual specie also known as American barnyardgrass because it is native to North America and is the most widespread specie in the United States (Bryson and Reddy 2012; Michael 2003; Yatskievych 1999). Rough barnyardgrass normally flowers in mid-summer to early fall. This weed is wind-pollinated and spreads by seeds. Rough barnyardgrass will thrive more in open, wet areas than in moist environments, and prefers loamy or silty fertile soil. This plant grows in floodplain woodlands, swamps, marshes, low areas along ponds and rivers, ditches, croplands, abandoned fields, low areas in vacant lots, gardens and moist waste areas; it prefers degraded wetland or disturbed areas with exposed fertile ground.

Species Description. Rough barnyardgrass can grow up to 160 cm and normally branches at the base. It can have multiple culms that will sprawl and stand erect, but these culms are unbranched. The glabrous culms are light green. It has alternate leaf arrangement with leaf blades that are up to 40.5 cm long, 0.8-30 cm wide and have tiny serrations on the margins

(Michael 1992; Gould et al. 1972; Holm et al. 1977). The leaf blades are green or blue-green in color and hairless. The base of the leaf blade may have some long hairs (Holm et al. 1977).

Each culm has a panicle that is about 7-35 cm long. The panicle has branches up to 2-8 cm long, which spread outward as the panicle matures. It has dense spikelet arrangement on one side of each branch. The spikelets are between 2.5-5 cm and light to dark green in color and covered with fine hairs. In bright sunlight, it can become purple or dark red (Holm et al. 1977). The upper glumes are the same size as the spikelets and hide the fertile lemma, which encloses the florets on one side. The lower glume is about 1/3 of the spikelet. The lower lemma is sometimes awnless or awned, which can be 3-10 mm long and sometimes up to 25 mm long (Michael 2003; Gould et. al 1972). The lower palea is well developed with whitish or purplish color (Gould et al. 1972). This specie and barnyardgrass, have small morphological differentiation. One difference is that the upper lemma of barnyardgrass has a fragile tip and a ring of small hairs at the base of this tip while rough barnyardgrass has a firm, flexible tip that is without hairs (Holm et al. 1977).

Dormancy as Weedy Trait

One of the most common attributes of many weed seeds is dormancy. Seed dormancy makes it difficult to predict accurately the timing and extent of the emergence of a weed. A seed is dormant when it fails to germinate under a condition that is favorable for its germination (Finch-Savage and Leubner-Metzger 2006). Dormancy is a weedy trait, which allows the weed to escape management tactics, or survive harsh environmental conditions. This trait allows the weed to persist long in the soil seedbank (Simpson 1990). Variability in seed dormancy is regulated by environmental and genetic factors such as temperature and after-ripening

requirement (Chauhan and Johnson 2009; Bagavathiannan et al. 2011). Information on the timing of germination of *Echinochloa* seeds can help the farmers estimate its emergence potential and, thus, adjust the crop planting date to favor rapid crop establishment and early growth. An understanding of the seed dormancy behavior in *Echinochloa* can also help us understand their adaptation mechanisms to a particular habitat.

Seed Dormancy in Echinochloa species

Dormancy is a seed survival mechanism that is controlled by multiple genes and is heritable (Kon et al. 2007). The expression of dormancy is mediated by hormones, abscisic acid, gibberellin, and ethylene; and is strongly influenced by environmental factors during seed formation and after maturity (Finch-Savage and Leubner-Metzger 2006). Crop production practices that may occur after weed maturity including irrigation, fertilizer application, or tillage, also influence weed seed dormancy (Kon et al. 2007).

Seed dormancy in *Echinochloa* spp creates a problem in weed management because the seed will germinate at different times, making weed control difficult (Martinkova 1989). Seeds can remain 100% viable for 6-8 years in dry condition (Maun and Barrett 1986); longevity in soil varies according to soil texture. *Echinochloa* species, specifically barnyardgrass, were reported to remain viable up to 13 years in sandy loam soil when buried at 20 cm depth (Dawson and Bruns 1975). In the study by Dawson and Bruns (1975), they buried the seeds at 2.5, 10 and 20 cm in Warden, Washington, USA and exhumed the seeds every other year to determine the germination capacity and seed viability (Dawson and Bruns 1975). Thorough understanding of germination behavior across *Echinochloa* species will enable proper management in the field to reduce infestation.

Seeds with shallow dormancy will have high germination capacity when exposed to suitable environmental conditions such as optimum moisture, temperature, and light (Vleeshouwers et al. 1995; Baskin and Baskin 1998; Finch-Savage and Leubner-Metzger 2006). Seed dormancy has been one of the main research topics on *Echinochloa* species in the last 67 years (Bewley and Black 1982), but this has been done mainly with *E. crus-galli*, which is believed to be the most important weed in the rice field. Little attention was given to the dormancy behavior of other *Echinochloa* species. Because of the abundance of junglerice and rough barnyardgrass in the southern United States, it is important to study the dormancy behavior of these species.

Dormancy in *Echinochloa* species is expressed after maturity; some seeds will still be dormant 3-5 months after maturity while some will remain dormant for up to 7 months (Honek and Martinkova 1996). Shallow physiological dormancy can be overcome by manipulating soil temperature, soil moisture, light, and soil nitrate or certain combinations of these factors (Benech-Arnold et al. 2000; Probert 2000). Dormancy-breaking treatments are species-specific and sometimes, even ecotype-specific (Donahue 2005; Grundy 2003; Finch-Savage and Leubner-Metzger 2006). Most researchers studied environmental factors or scarification methods to release dormancy, but interspecies variation in dormancy has not been evaluated (Finch-Savage and Leubner-Metzger 2006). It is common to have more than one species in a field. The co-occurrence of multiple species presents a bigger problem in weed management because of dormancy variation, resulting in the intermittent emergence of *Echinochloa* throughout the crop-growing season. This also favors selection for resistance to herbicides as individuals escape herbicide applications due to variable age and size, or by merely emerging much earlier or later.

Secondary dormancy can occur after termination of primary dormancy, but this will only happen if the seed is exposed to unfavorable conditions (high or chilling temperature, absence of light, water stress) for some time. Barnyardgrass normally has 5-50% non-dormant seed that can germinate soon after maturation; the rest will stay dormant and remain in the soil seedbank for a long time (Honek and Martinkova 1996). A study by Kon et al. (2007) has confirmed further that only 39% of new barnyardgrass seed germinate in the field.

Light Effect on Dormancy

Light is an important factor for germination, but light requirement varies across species (Chauhan and Johnson 2009). Germination of freshly harvested junglerice seed was 43% in alternating light/dark condition and only 4% under total dark condition. Complete darkness induces secondary dormancy in coast cockspurgrass (Kovach et al. 2010). Coast cockspurgrass seed goes into deep dormancy when buried deep in the soil profile where the seed can no longer perceive light (Kovach et al. 2010).

Temperature and Light Interaction Effect on Dormancy

Echinochloa, in general, requires a minimum temperature of 13 °C to germinate, reaches optimum germination between 20 °C and 30 °C, and achieves maximum germination at 40 °C (Brod 1968). Roche and Muzik (1964) studied the germination of barnyardgrass at 30 °C without light, 20-30 °C alternating temperature (20 °C for 16 h and 30 °C for 8 h) with light, 20 °C with continuous light, 15-20 °C alternating temperature (15 °C for 16 h and 20 °C for 8 h) without light, 15 °C without light, and 10 °C without light. They observed that the highest germination was achieved at an alternating temperature of 20 °C for 16 h and 30 °C for 8 h with

light. Germination did not differ between storage conditions in room temperature and in an unheated building. Barnyardgrass did not germinate for six weeks when incubated at 10 °C.

Seed Size and Seed Position Effect on Dormancy

Seed weight is associated with length of dormancy where larger seed tends to have longer dormancy period than the smaller seed (Baskin and Baskin 1998). This is because the bigger seed has more starch reserve (energy source) than the smaller seed. The individual seed weight varies depending on the seed position on the panicle (Chun and Moody 1986). The seed maturity level also varies across the length of the panicle. Thus, dormancy level varies according to the position of the seed on the panicle. In junglerice, the second spike produces the least number of seed and is the shortest (Chun and Moody 1986). The spike length and number of seeds per spike gradually increase towards the base of the panicle while the germination capacity and seed weight decrease towards the bottom of the panicle. In other words, seeds at the bottom of the panicle are more dormant than those toward the top. The lower spikes also contain a higher percentage of sterile seeds (Chun and Moody 1986).

Roche and Muzik (1964) also compared light-colored (1.42 g for 1000 seeds) and darkcolored seed (1.94 g for 1000 seeds) under an alternating temperature of 20 °C for 16 h and 30 °C for 8 h. The dark-colored, larger seed was less dormant than the small, light-colored seed. In the field, *Echinochloa* species attained only about 39% germination in a 6-month period (July-December) (Kon et al. 2007). This is a small fraction of the seed added to the seedbank, which results in a net increase of soil seedbank size across seasons. The extended germination behavior will also be a challenge for farmers who are not capable of expending a lot of input for seasonlong weed management. The efficacy of cultural control practices, such as stale seedbed

approach, requires accounting for variations in time to seedling emergence (Fischer et al. 2009), which is dependent upon the dormancy status of seeds (Vleeshouwers and Kropff 2000). Therefore, information on inter-species variation in germination behavior of barnyardgrass, rough barnyardgrass, junglerice or other weedy species will be useful in achieving reduction of the soil seedbank and effective weed control.

Similar Studies on Morphological Variation of Echinochloa species

Damalas et al. (2008) studied the morphological variation among 12 populations of *Echinochloa* species (barnyardgrass, early watergrass, and late watergrass) in the same environment in Greece. The seeds were collected from rice fields and tested for dormancy eight months after harvest. The seeds were germinated at 25 °C with 16 h of light. In that study, barnyardgrass showed the lowest germination capacity (16% in 7 days, 28% in 14 days, and 36% in 21 days) compared to early watergrass, which had the highest germination (53.25% in 7 days, 91.5% in 14 days, and 92.25% in 21 days). Late watergrass had higher germination (37.5% in 7 days, 68% in 14 days and 78.28% in 21 days) compared to barnyardgrass. This showed that barnyardgrass is more dormant than the two watergrass species. Damalas and colleagues (2008) also conducted two years of field experiment on the morphology of these 12 populations in one location. In their study, barnyardgrass had leaf length ranging from 40-44 cm, leaf width of 1.23-1.29 cm, height of 136-145 cm and 115-131 tillers per plant. The seed weight was 1.92-2.14 g/1000 seeds, spikelet length was 1.58-1.76 mm and spikelet width was 1.37-1.44 mm. Panicle initiation occurred 39-45 days after planting. The growth habit of *Echinochloa* changes with plant density, assuming a different canopy structure at high plant density, to optimize light interception (Damalas et al. 2008).

Tabacchi et al. (2006) sought to verify the reliability of taxonomic description by Pignatti (1982) and Carretero (1981) to differentiate Echinochloa species and to identify key traits that allow differentiation of *Echinochloa* species in Italian rice fields. In their research, 80 *Echinochloa* accessions were collected from rice fields in Italy, germinated in the greenhouse, transplanted to the field at 3-4 leaf stage, and characterized morphologically. Their data separated the accessions into two clusters using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The first cluster contained 46 accessions, which were identified to be barnyardgrass based on Pignatti's key; however, based on Carretero's key, the cluster was composed of a mixture of 30 barnyardgrass accessions and 16 E. hispidula accessions. The first cluster was composed of barnyardgrass and E. hispidula based on their red or purple-red basal stem section and spikelet size shorter than 3.6 mm. The second big cluster contained plants with green stem and spikelets longer than 3.9 mm. This cluster was further split into two subclusters. The large subcluster contained 32 accessions identified as E. erecta (23 accessions) and nine accessions of *E. phyllopogon* with hairy leaf sheath. The small subcluster contained only 2 accessions, one was identified as barnyardgrass and the other as E. oryzoides with awned spikelets, a glume/lemma ratio lower than 0.45 and absence of hairs on the collar region. Principal component analysis confirmed that these two main clusters were separated based on basal stem color (red and green). This study also indicated that 30% of total variability was explained by stem color and spikelet length. They concluded that the specific identification key for *Echinochloa* in Italy are as follows:

Cluster 1

- 1. Red basal stem and spikelet length less than 2.5 mm...... E. colona
- 2. Red basal stem, spikelet length between 3-3.6 mm E. crus-galli

Cluster II...green basal section

1.	Leaf sheath with hair	. E. phyllopogon
2.	Leaf sheath hair absent, awn longer than 1 cm and	
	glume/lemma ratio less than 0.45	. E. oryzoides
3.	Leaf sheath hair absent, awnless and ratio glume/lemma	
	more than 0.45	E. oryzicola

The Importance of Echinochloa Characterization

The biology of resistant weeds is a major focus of many weed scientists. Understanding the biological processes that influence resistance will help to reduce the evolution of resistance in weed populations, and understanding gene flow among resistant weedy species will help create models that predict the population dynamics of the herbicide-resistant weed (Roush et al. 1990).

It is important to characterize *Echinochloa* species because it is reported that *Echinochloa* species are becoming a late-season weed problem in crop production and are evolving multiple resistance to herbicides. Characterization will provide information on the occurrence of *Echinochloa* species in Arkansas (with the broader implication in the southern United States and elsewhere). It will allow us to make inferences on the distribution of species and will determine the extent of inter- and intra-specific morphological differences that may affect the efficacy of weed control measures. For example, Bryson and Reddy (2012) reported that junglerice do not necessarily have purple stripes on leaves, which is the principal trait practitioners use to identify this specie. The color of inflorescence also varies from green to purple. There is a need to find other traits to distinguish this specie correctly from *E. muricata* and *E. crus-galli*, which have

minimal differences in morphology. Further, phenological characterization of *Echinochloa* species is needed to determine growth stage variations between species and rice.

As mentioned before, junglerice was reported to have resistance only to glyphosate in California and multiple resistance to imazethapyr, propanil, and quinclorac in Arkansas. The abundance of this specie in crop production in the southern United States, and the increasing reports of herbicide resistance on *Echinochloa* species everywhere in the southern United States, creates doubt whether resistance to herbicides is correctly attributed to junglerice or barnyardgrass (Heap 2015). Junglerice is widespread in the southern United States, but no herbicide resistance was attributed to this specie. Further, because the literature indicates that there is little morphological variation between rough barnyardgrass and barnyardgrass (Hitchcock 1951); it is possible that *Echinochloa* species are misidentified often. Proper identification is needed to understand the differential response to herbicides and to improve weed management recommendations for *Echinochloa* species. Species and ecotype diversity contribute to variation in efficacy of weed management tactics. Hence, the present study will help in species identification not only for Arkansas but also for other regions.

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CHAPTER III

CHARACTERIZATION AND IDENTIFICATION OF ECHINOCHLOA SPECIES IN

ARKANSAS, USA

Abstract

- **Premise of the study**: *Echinochloa* comprises many weedy species, several of which had evolved resistance to herbicides. Research was conducted to determine relative species abundance and characterize the diversity in weedy traits of *Echinochloa* at the Arkansas Agricultural Research and Extension Center, Fayetteville, AR, USA.
- Methods: Accessions were grown as a single plant in a common garden, in a randomized complete block design (RCBD) with four replicates in two years. Twenty vegetative and reproductive traits pertaining to the culm, leaf, panicle, spikelet, and seed production were measured. Seedling emergence, flowering time, and days to maturity were also recorded.
- **Key results:** Junglerice (*Echinochloa colona*) was the most abundant species, comprising 78% of the accessions collected. Barnyardgrass (*Echinochloa crus-galli*) and rough barnyardgrass (*Echinochloa muricata*) comprised about 9% and 12% of the accessions, respectively. Junglerice was the shortest (65-94 cm) and rough barnyardgrass was the tallest (79-118 cm) of the three species. Rough barnyardgrass had the longest (19.8-42 cm) and widest (1.2-2.1 cm) flag leaves and flowered the latest, at 46-63 days after emergence (DAE). Junglerice and barnyardgrass initiated flowering 39-59 DAE. Junglerice produced the highest number of seed (9098-217217 per plant), with an average of 539 seeds per panicle. Barnyardgrass produced fewer seeds (7186-71494 seed/plant), with an average of 317 seeds per panicle. The growth habit of junglerice could be either prostrate, decumbent, or open whereas that of barnyardgrass and rough barnyardgrass could be decumbent or open.

• **Conclusions:** Junglerice and barnyardgrass were most similar in vegetative and panicle characteristics and color diversity, resulting in misidentification. Response to herbicides and other management practices may vary between species; thus, proper identification is helpful in achieving sustainable *Echinochloa* management.

Key words: barnyardgrass; coast cockspurgrass; distribution; *Echinochloa* species; emergence; growth habit; junglerice; morphology; phenology; rough barnyardgrass,

Echinochloa is among the top ten world's worst weeds in terms of area infested, management difficulty and yield losses (Holm et al., 1977). Most of the species in this genus are adapted to both tropical and warm temperate climates; several species are weedy and grow well in flooded rice fields or in non-flooded crop areas, causing significant yield losses to rice and other crops (Smith, 1983; Maun and Barrett, 1986; Lopez-Martinez et al., 1999). Echinochloa species are among the most difficult taxa to identify because the species tend to intergrade, causing misidentification (Yatskievych, 1999). Identification is further complicated by the limited comprehensible monographic treatment and some introduced Echinochloa species could hybridize with native ones (Maun and Barrett, 1986). Interspecific hybridization increases the genetic diversity of the species complex in this genus, endowing greater potential for adaptation to abiotic and biotic stresses in natural and managed ecosystems (Simberloff, 2000; Salej et al., 2014). Increased species diversity also favors the evolution of resistance to herbicides, which are the main tools used to manage weedy species in agronomic crops (Owen and Zelaya, 2005). Species and ecotype diversity favor population shifts, adaptation to cultural management practices, or localized adaptations to agro-ecological environments (Roy et al., 2000).

Echinochloa is the most important weed in rice production in Arkansas and the southern US rice belt (Dowler, 1995; Norsworthy et al., 2013). It is agronomically and economically important to identify *Echinochloa* species properly because weedy *Echinochloa* can invade and dominate a crop field in just one season and cause significant crop losses (Ruiz-Santaella et al., 2006). The morphological diversity of *Echinochloa* species also signifies potential differences in response to crop and weed management practices.

Lately, the evolution of resistance to herbicides in the *Echinochloa* complex is rendering an increasing number of herbicide groups ineffective. Barnyardgrass [Echinochloa crus-galli (L.) Beauv.], one of the most problematic species under the *Echinochloa* species has evolved resistance to ten herbicide groups including 1) Group 1, inhibitors of acetyl coenzyme-A carboxylase (ACCase); 2) Group 2, inhibitors of acetolactate synthase (ALS); 3) Group 3, Microtubule inhibitors; 4) Group 4, synthetic auxin; 5) Group 5, inhibitors of Photosystem II; 6) Group 7, inhibitors of Photosystem II (urea and amides); 7) Group 8, lipid inhibitors; 8) Group 13, carotenoid biosynthesis inhibitors, 9) Group 15, inhibitors of long chain fatty acids; and 10) Group 26, inhibitors of cellulose synthesis in 22 countries (Heap, 2015). Resistance to multiple modes of action was reported in eight cases. Junglerice [Echinochloa colona (L.) Link] is reported to have resistance to six herbicide groups including 1) Group 1; 2) Group 2; 3) Group 4, 4) Group 5; 5) Group 9, inhibitors of 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase 6) Group 26 in 14 countries including 5 multiple resistance (Heap, 2015). The most widely distributed species in North America, rough barnyardgrass [Echinochloa muricata (P. Beauv.) Fernald], might have been overlooked in reporting herbicide resistance (Michael 2003; Bryson and Reddy, 2012). This could be due to the mixing of rough barnyardgrass with barnyardgrass in resistance testing, or the relatively low population density of rough barnyardgrass in rice fields

such that it is overlooked in the sampling process. In Belgium, rough barnyardgrass has been reported to have reduced sensitivity to ALS-inhibitor herbicide (nicosulfuron) and HPPD-inhibitor (topramezone, mesotrione, tembotrione and sulcotrione) (Cauwer et al., 2011). In Arkansas, barnyardgrass has been reported to have resistance to propanil, quinclorac, clomazone and imazethapyr (Riar et al., 2015). A survey by Norsworthy et al. (2013) showed that 50% of barnyardgrass populations tested have resistance to propanil and about 25% to quinclorac. About 20% of the quinclorac-resistant populations are also resistant to propanil.

Sampling of *Echinochloa* between 2010 and 2013 from rice and soybean fields in the Mississippi River Delta in Arkansas, USA indicated that the most abundant species is junglerice (Burgos, University of Arkansas, personal communication). This is consistent with the report by Bryson and Reddy (2012) on the relative abundance and distribution of *Echinochloa* in the southern United States. It is, therefore, possible that resistance to herbicides in junglerice has been under-reported or that it has been attributed to barnyardgrass.

The growth habit and other morphological features of *Echinochloa species* are strongly affected by environmental conditions such as plant population density, soil texture, soil fertility, temperature/relative humidity, and others. In many occasions, local adaptations have resulted in the evolution of different ecotypes within a species (Yoshioka et al., 1998; Lopez-Martinez et al., 1999). *Echinochloa* accessions showed distinct variations in morphological traits when it is sampled 10 km apart; this indicates the existence of ecotypic differentiation even in the same locality (Barrett, 1992; Yamasue, 2001; Yoshioka et al., 1998). The present study aimed to characterize the morphological and phenological diversity of predominant *Echinochloa* species in Arkansas, USA; and identify traits that can be used to distinguish among species and which could potentially contribute to differentiation in weediness between species.

MATERIALS AND METHODS

Plant materials—Ninety-four accessions of *Echinochloa* were collected between 2010 and 2011 from rice and soybean fields in 23 counties in Arkansas, USA (Table 3.1). In this research, 'accessions' refer to a composite sample of seeds collected from at least 20 plants, of identical appearance, per field. Because rice and soybean are most commonly planted in rotation (Burgos et al., 2008), *Echinochloa* infesting rice and soybean fields are exposed to the same agronomic practices, such as the types of herbicides used, irrigated or non-irrigated crop culture during soybean production and flooded crop culture during rice production. Fields, along rural roads, with *Echinochloa* infestation in the late season, were sampled. The intent was to represent all rice-producing counties in the State with one or more samples from each rice-producing county. The geographical coordinates of each collection site were marked using the global positioning system. The seeds were air-dried, cleaned, placed in paper envelopes, cataloged, and stored at room temperature.

Characterization of accessions in a common garden—Field experiments were conducted at the Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013. The soil was a Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudults). Soil samples collected from the field in 2012 and 2013 were analyzed at the Soil Testing Laboratory, University of Arkansas, Fayetteville, USA. In 2012, the field used had 18% sand, 70% silt and 11% clay. The soil pH was 6.1, cation exchange capacity (CEC) was 11 cmol⁺/kg, Mehlich-3 extractable phosphorous was 83 mg/kg and potassium was 103 mg/kg (Appendix 3.1). The field soil in 2013 had 25% sand, 63% silt and 12% clay. The soil pH was 5.6, CEC was 9 cmol⁺/kg, Mehlich-3 extractable phosphorous was 66 mg/kg and potassium was

87 mg/kg (Appendix 3.2). The daily temperature mean in 2012 ranged from 16.8-30.3°C with 46 d above 32.3°C. In 2013, the mean temperature ranged from 17.5-32°C with 61 d above 32.3°C (NOAA, 2014). These temperatures were within the optimum range for the growth of *Echinochloa* species (Lopez-Martinez et al., 1999).

The experimental design was a randomized complete block with four replications. In 2012 and 2013, 94 accessions were characterized (Table 3.1). Seeds of each accession were planted in 9 cells in a 50-cell tray [self-watering capillary tray] filled with Sunshine Mix#1 potting medium [Sun Gro Horticulture, Inc. Bellevue, Washington, US]. Seedlings were raised in the greenhouse with 32/22°C day/night temperature from June 20, 2012 and June 14, 2013. The trays were sub-irrigated. Two weeks after planting, seedlings were thinned to two per cell. At the 4-leaf stage (about 3 weeks after planting), seedlings were transplanted manually to the field in a 1.5 m by 1.5 m spacing. Transplanting occurred on July 5, 2012 and June 28, 2013. In 2012, the whole field was sprayed with a mixture of glyphosate (1.12 kg ae/ha) and S-metolachlor (1.79 kg ai /ha) 7 days before transplanting to control other weeds. The field was watered when needed and kept weed-free by hoeing other weeds. In 2013, a mixture of pendimethalin (1.0 kg ai/ha) and S-metolachlor (1.0 kg ai/ha) was sprayed 7 d after transplanting to control other weeds. Nitrogen fertilizer (60 kg N/ha) was applied on the day of transplanting at by incorporating the fertilizer around the base of the plant. Insecticide (imidacloprid, 0.22% granule) was applied at 0.20 kg ai/ha to control wireworms.

Days to initiation of emergence were recorded in the greenhouse and days to heading were recorded in the field when 50% of tillers had exerted its panicle. The number of days to heading and days to maturity were calculated from the day of emergence (DAE) of each accession. Plant height, number of tillers, and length and width of flag leaf were measured 82

days after planting (DAP). Plant height was measured from the ground to the longest point of the panicle on the tallest tiller. The length and width of flag leaf were recorded from four tillers per plant. Leaf width was measured at mid-length of the leaf blade while leaf length was measured from the collar to the tip. Color of leaf sheath, leaf blade, internode, and node was recorded at 82-90 DAP. Flag leaf angle was estimated relative to the vertical axis at 82 DAP. For example, a horizontal leaf has an angle of 90° and an erect leaf has an angle of 0-15°. Growth habit was recorded in four categories: open, decumbent, and prostrate. Reproductive traits, evaluated at maturity, included days to maturity of 50% of panicles, number of panicles and panicle length (measured from the first branch to the tip of panicle). Hull color was recorded at seed-filling, before the glume and lemma (hull) turned brown. Awn length was measured in the field using a 15-cm digital caliper (Neiko Stainless Steel) [Neiko Tools USA (Amazon), Seattle, Washington]. Length and width of spikelet, length of upper glume, lower glume, lower lemma, upper lemma and panicle branch length were measured in the laboratory, with the aid of a dissecting microscope [Amscope, 14370 Myford Road, #150, Irvine, CA 92606], using Neiko Stainless steel 15-cm digital caliper. The upper glume, lower glume, upper lemma and lower lemma were measured using digital veneer caliper at the center of each tissue. Two spikelet per plant from the middle third of the panicle were used for this characterization.

Mature panicles were harvested and air-dried for 7 d. A subset of 10 panicles per plant were threshed and cleaned to remove sterile florets. Filled seeds from these 10 panicles were weighed and the 500 seed weight was recorded. Seeds were counted using Elmor C1 seed counter [Firma Elmor, Mangelegg 58, 6430 Schwyz, Schweiz, Switzerland]. The remaining panicles were also threshed and the weight of seeds recorded. The total number of seeds per

plant was estimated based on the total seed weight from the representative panicles harvested, multiplied by the total panicles per plant.

Species identification— In 2013, a representative plant (culm, leaf, and panicle) from each accession was harvested and pressed using a Bioquip herbarium plant press and mounted on 100% cotton white herbarium mounting paper (University of California-Davis, USA standard), 28.5 cm x 41 cm in size [Herbarium Supply, 705 Bridger Drive, Unit D, Bozeman, Montana, USA]. The herbarium collection is kept at the Altheimer Laboratory, University of Arkansas, Fayetteville, USA for future reference. Data recorded both in the field and in the laboratory were compared with literature and used to identify the species of each sample with reference to the treatments of Gould et al. (1972), Yatskievych (1999) and Michael (1983, 2001, 2003). Vouchers from the Intermountain Herbarium, Utah State University and from the Herbarium of the University of Arkansas, Fayetteville were also used as a reference for species identification. The vouchers were labeled with common name, scientific name, county where it was collected and the year it was collected.

Statistical analysis—All data except caryopsis color, leaf sheath color, leaf blade color, node color, inter-node color, leaf angle, and growth habit, were subjected to analysis of variance (ANOVA) and was pooled for analysis in the absence of a significant year effect. All statistical analyses were performed using JMP for Windows software (Version 12.1; 2015, Statistical Analysis Systems Institute, SAS Circle, P.O. Box 8000, Cary, NC 25712-8000). Differences among species in quantitative traits were tested using one-way ANOVA and means were separated by Fisher's Protected LSD test with alpha level 0.05. Frequency analysis was also

done to evaluate species differences in caryopsis color, leaf sheath color, leaf blade color, node color, inter-node color, flag leaf angle and growth habit.

Cluster analysis—Cluster analysis, using all plant traits, was conducted to verify species identity. The accessions were grouped using cubic clustering criterion. The number of clusters in the dataset was identified when cubic clustering criterion reached a maximum with every 1-point increment in the number of clusters, and decreasing thereafter.

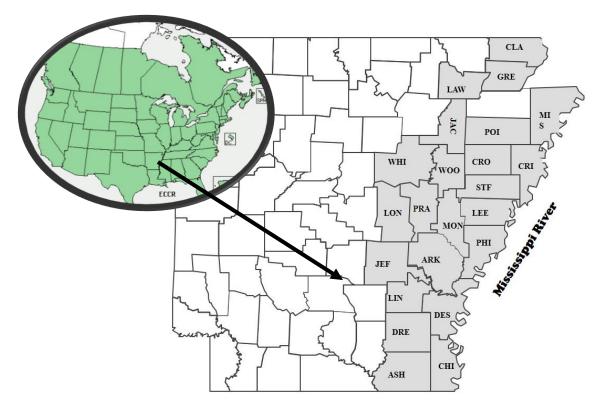


Figure 3.1 Collection sites of Echinochloa accessions in 2010 and 2011, Arkansas, USA.

RESULTS AND DISCUSSION

Species Composition—Of the 94 accessions, 73 were identified as junglerice, 9 as barnyardgrass, 11 as rough barnyardgrass and 1 as coast cockspurgrass [Echinochloa walteri (Pursh) A. Heller] (Table 3.1). However, these species were not identified up to the variety level because the high background variability within Echinochloa species masks the smaller differences among varieties (Yatskievych, 1999). This makes variety identification unreliable. Accessions identified as junglerice were collected from the following counties: Arkansas, Ashley, Chicot, Clay, Crittenden, Cross, Desha, Drew, Greene, Jackson, Jefferson, Lawrence, Lee, Lincoln, Lonoke Mississippi, Monroe, Phillips, Poinsett, Prairie, St Francis and White. The presence of junglerice in Clay, Cross, Greene, Jackson, Jefferson, Lawrence, Lee, Lonoke, Monroe, Phillips, Poinsett, St Francis, White and Woodruff counties was not reported in the previous survey by Gentry et al. (2013) and Smith (1988). Barnyardgrass accessions were sampled from Ashley, Jackson, Lawrence, Lincoln, Mississippi, Monroe, Prairie and White counties. The presence of barnyardgrass in White county was not reflected in the work of Gentry et al. (2013) and Smith (1988). Rough barnyardgrass accessions were sampled from Clay, Greene, Jackson, Lawrence, Mississippi, Poinsett and St. Francis counties. The only sample identified to be coast cockspurgrass was collected from Chicot County.

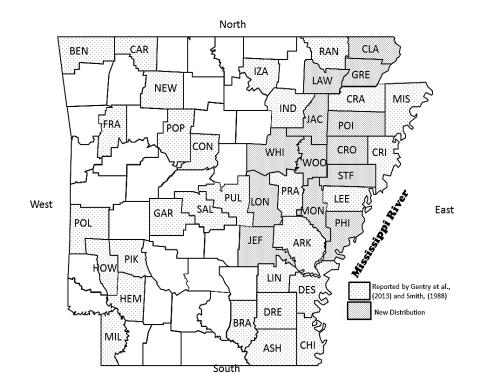
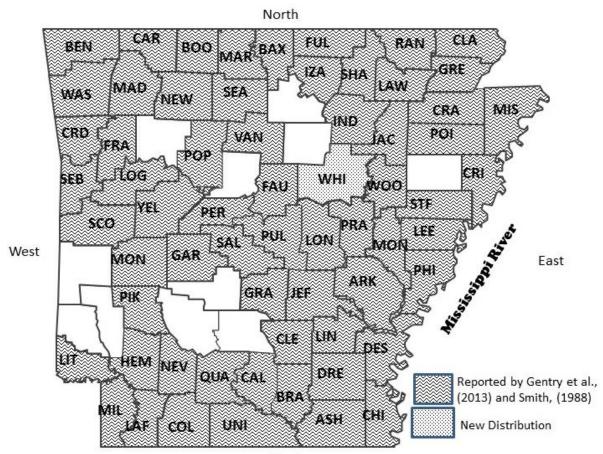
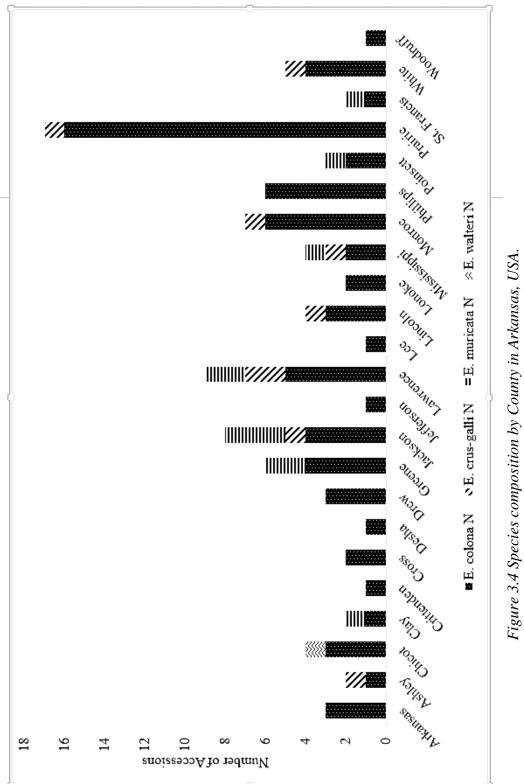


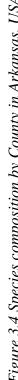
Figure 3.2 Updated distribution of junglerice in Arkansas, USA. County Code BEN = Benton, CAR = Carroll, NEW = Newton, IZA = Izard, CRA = Craighead, POI = Poinsett, MIS = Mississippi, CRI = Crittenden, LEE = Lee, BRA = Bradley, DRE = Drew, LIN = Lincoln, ARK = Arkansas, DES = Desha, CHI = Chicot, HEM = Hempstead, MIL = Miller, PIK = Pike, PRA = Prairie, HOW = Howard, IND = Independence, POL = Polk, GAR = Garland, Pulaski, POP = Pope, CON = Conway, RAN = Randolph, SAL = Saline, FRA = Franklin, CLA = Clay, GRE = Greene, LAW = Lawrence, JAC = Jackson, POI = Poinsett, CRO = Cross, STF = St. Francis, WOO = Woodruff, WHI = White, LON = Lonoke, MON = Monroe, PHI = Phillips, JEF = Jefferson



South

Figure 3.3 Updated distribution of barnyardgrass in Arkansas, USA. County Code BEN = Benton, CAR = Carroll, BOO = Boone, MAR = Marion, BAX = Baxter, FUL = Fulton, SHA = Sharp, RAN = Randolph, CLA = Clay, GRE = Greene, WAS = Washington, MAD = Madison, NEW = Newton, SEA = Searcy, IZA = Izard, SHA = Sharp, LAW = Lawrence, CRA = Craighead, POI = Poinsett, MIS = Mississippi, CRI = Crittenden, JAC = Jackson, IND = Independence, VAN = Van Buren, POP = Pope, FRA = Franklin, CRD = Crawford, SEB = Sebastian, LOG = Logan, YEL = Yell, PER = Perry, FAU = Faulkner, WOO = Woodruff, STF = St. Francis, LEE = Lee, MON = Monroe, PRA = Prairie, LON = Lonoke, PUL = Pulaski, SAL = Saline, GAR = Garland, MON = Montgomery, PIK = Pike, LIT = Little River, HEM = Hempstead, NEV = Nevada, OUA = Ouachita, CAL = Calhoun, DRE = Drew, GRA = Grant, JEF = Jefferson, LIN = Lincoln, ARK = Arkansas, DES = Desha, CHI = Chicot, MIL = Miller, LAF = Lafayette, COL = Columbia, UNI = Union, ASH = Ashley, WHI = White





Thus, junglerice is the most abundant species in rice and soybean fields in the Mississippi River Delta in Arkansas, USA. These findings are consistent with the report of Bryson and Reddy (2012) stating that the most common *Echinochloa* species in the southern United States is junglerice. The second most common species is rough barnyardgrass, which conforms to the research by Smith (1988). This indicates that junglerice is the primary threat to rice production in Arkansas.

Emergence characteristics— The seedling emergence period did not vary significantly among species, all of which showed a wide range in days to initiation of emergence (Table 3.2). Junglerice and barnyardgrass emerged within 2-8 DAP. Arifin et al. (2004) also reported in an earlier study that barnyardgrass emerges between 2.8 and 7.2 DAP. Despite the high intraspecies variability in emergence, which masked differences between species, rough barnyardgrass accessions were generally the latest to emerge. The diversity in emergence behavior across barnyardgrass and junglerice populations presents a big problem for weed management because these species can emerge across a wide window of time, allowing many to escape weed management tactics (Scursoni et al., 2007).

Vegetative Characteristics—Plant height differed across species, but barnyardgrass and rough barnyardgrass were equally tall (79.1-125.5 cm) (Table 3.2). In contrast, Barrett and Wilson (1983) reported barnyardgrass to be 19% taller than rough barnyardgrass. Rough barnyardgrass and barnyardgrass were within the range described by Michael (2003) and Gould (1976). Coast cockspurgrass was excluded from the analysis because data for this specie were obtained from only one accession (Table 3.2). This specie was encountered in only one sampling site. It was

tall like the rough barnyardgrass (Michael, 2003). Junglerice was the shortest species compared to barnyardgrass and rough barnyardgrass. Junglerice accessions from Arkansas were slightly taller than those described by Michael (2003). Whereas plants in these experiments were grown as separate individuals in the same weed-free environment, the plants characterized by Michael (2003) were most likely growing in the field with a crop or other weeds.

Junglerice produced significantly more tillers than rough barnyardgrass and had the greatest number of tillers among *Echinochloa* species (Table 3.2). The high tillering capability allows junglerice to produce a large number of panicles (and seeds), which contributes to its abundance. In rice fields, competition with rice drastically reduces number of tillers to generally less than 10 productive tillers per plant (Burgos, N.R. pers. observation). However, where there are gaps in crop rows and in field edges, *Echinochloa* species can easily reach its tillering potential and maximum seed production. Barnyardgrass in this study produced fewer tillers, with high variability in tillering than barnyardgrass in Greece (115-131 tillers) (Damalas et al., 2008). Unless direct comparison is made in one experiment, it is unknown how much genotype and environment contribute to such intraspecific differences across different world regions. It is possible that the barnyardgrass in Greece is a high-tillering ecotype compared to the barnyardgrass in the southern USA. In which case, farmers in Greece are expected to incur higher crop yield losses due to barnyardgrass competition than farmers in the United States.

Rough barnyardgrass had the longest flag leaf among the three species, significantly longer than junglerice and barnyardgrass (Table 3.2). The flag leaf of junglerice was slightly longer than was described by Michael (2003) whereas the flag leaves of barnyardgrass and rough barnyardgrass were within the range described by Michael (2003). Rough barnyardgrass had the widest flag leaves compared to junglerice and barnyardgrass. Big flag leaves provide a

competitive advantage because of large surface area for light interception, which equates to higher photosynthesis capacity. A big plant such as rough barnyardgrass needs to fix a large amount of carbon to develop such a large plant canopy and biomass. Also, big flag leaves afford higher capability to shade adjacent plants and gain competitive advantage (Weiner and Thomas, 1986).

Green leaf sheath color was common among the three species (Table 3.3). Junglerice and rough barnyardgrass had a mixture of green and purple (purple with green in the middle) leaf sheath of 7% and 18%, respectively. Barnyardgrass and junglerice also had 11% and 1% of their accessions, respectively, with light green leaf sheath. Purple nodes color was another common trait in all three species. Among junglerice accessions, 37% had purple nodes, 33% had light green nodes, 18% green, 11% light purple and 1% green-purple node (purple on both end with green in middle of the node). In barnyardgrass, 67% of the populations had purple nodes, 22% had green and 11% had light green nodes. In rough barnyardgrass, 37% had purple nodes, 27% had light green nodes and 18% respectively, had green-purple mixture nodes and green nodes. Most of the junglerice (45%), barnyardgrass (67%) and rough barnyardgrass (55%) had purple and green mixture internodes (green internode with purple edge). The second most common inter-nodes color for junglerice is light purple (30%) and purple for rough barnyardgrass (27%). In junglerice, 11% of the accessions had purple internodes, 8% had green internodes and 5% had light green internodes. In barnyardgrass, light green, purple and light purple internodes had equal percentages at 11% each. In rough barnyardgrass, there were an equal proportion of plants (9%) with green and light purple internodes.

Some difference in leaf pigmentation was observed among and within species. This is usually a response to environmental cues (Gould et al., 1995). There is no information whether

photosynthesis is higher in plants of the same species with green leaves than in plants with purple leaf pigmentation. Plants with purple leaf pigmentation were observed in all species (Table 3.3). The majority had purely green leaf blade with junglerice at 93%, barnyardgrass at 89%, and rough barnyardgrass at 82%. Green leaves with purple margin were commonly observed in rough barnyardgrass (18%), which helps to differentiate it from barnyardgrass. Green with purple margin leaf blade was observed only in rough barnyardgrass. Light green leaf blades were observed in barnyardgrass (11%) and junglerice (7%).

Leaf angle is one characteristic that contributes to light interception (Damalas et al., 2008). Erect leaves, e.g. starting from 45° angle, allow maximum light penetration to the lower canopy. As leaves are positioned closer to the horizontal, light penetration to the lower canopy decreases and the productivity of lower leaves is reduced. About 48% of junglerice leaves were relatively erect (generally with 45° angle), indicating an efficient light-harvesting canopy structure (Table 3.3). This is very helpful for a relatively small and short-statured species like junglerice. Barnyardgrass (67%) and rough barnyardgrass (55%) had nearly horizontal leaves. Because these plants were tall and had larger leaves and fewer tillers than junglerice, an open positioning of leaves allows maximum shading of adjacent plants (Weiner and Thomas, 1986).

Growth habit is strongly affected by environment (Lopez-Martinez et al., 1999; Yoshioka et al., 1998). Barnyardgrass (56%) and junglerice (52%) typically had open canopy architecture, which allows maximum light penetration (Table 3.3). The majority of rough barnyardgrass (73%) had a decumbent growth habit, which allows this species to have the largest canopy coverage compared to barnyardgrass and junglerice. Erect growth habit is typical of plants growing in a dense population (Van Hinsberg, 1997); such canopy structure optimizes light penetration down the canopy to the lower leaves. Erect growth, coupled with narrow leaves and

shorter plants are adaptive traits that mimic the rice growth habit (Yamasue, 2001) and allows *Echinochloa* to escape weeding operations.

Reproductive characteristics— Junglerice and barnyardgrass flowered at the same time on average and earlier than rough barnyardgrass (Table 3.2). Based on heading data, junglerice and barnyardgrass in Arkansas need to be controlled in the first 49 DAE to prevent seed production. To minimize or prevent competition, weed control needs to be done earlier. The critical period to control barnyardgrass is between 15 and 30 DAP in direct-seeded rice (Azmi, 1990). In Arkansas, a barnyardgrass infestation of 100 plant/m² can reduce the yield of 'Newbonnet' rice planted at a density of 219 plant/m² by 10% in 17 days of interference, 20% in 34 days, 50% in 86 d and 70% in 120 d or season-long interference (Smith, 1993). Rough barnyardgrass accessions flowered between 42 and 63 DAE. This, together with the later tendency to emerge, also suggests that rough barnyardgrass will compete with rice for a longer period than barnyardgrass and junglerice and will be a problem later in the growing season. Fields infested with rough barnyardgrass require continuous scouting and targeted weed control measures, which is difficult to implement. The flowering periods of barnyardgrass observed in this study were longer to what was reported by Damalas et al. (2008). In their work, barnyardgrass flowered within 39-45 DAP. There were significant differences in days to maturity among species. Junglerice was the earliest to mature followed by barnyardgrass. Rough barnyardgrass accessions generally matured the latest. The period to maturity overlaps significantly across species, allowing for interspecies hybridization to occur in the case of genetic compatibility. Barnyardgrass have a close relation to coast cockspurgrass based on the nuclear DNA internal transcribed spacer (ITS) regions and it is also suggested that coast

cockspurgrass is the male parental origin of barnyardgrass (Aoki and Yamaguchi, 2008). Barnyardgrass and rough barnyardgrass also have strong potential to produce hybrids when growing in the same community (OLA and MAFF, 2002).

Panicle counts differed between junglerice and barnyardgrass (Table 3.2). Rough barnyardgrass and coast cockspurgrass produced the fewest panicles. Panicle lengths were significantly different among species, which could be a reflection of plant size (r (93) = 0.603, p<0.01). Rough barnyardgrass had the longest panicles followed by barnyardgrass. Junglerice had the shortest panicles at 10.5-19.2 cm. Junglerice in Arkansas had longer panicles than those in Missouri (5-12 cm) (Yatskievych 1999), although plants from both regions need to be grown in the same environment to verify this. If true, this indicates that junglerice in Arkansas could produce more seeds than the ecotype in Missouri. This is a plausible expectation because Missouri is on a more northern latitude relative to where the Arkansas accessions were collected. Research by Chun and Moody (1987) on 12 junglerice ecotypes collected from different habitats showed that the number of days to flower was correlated with latitude. Plants from higher latitude flowered 12 d earlier than plants from lower latitude. There were also significant differences across latitudes in spikelet size and total seed production. However, Chun and Moody (1987) did not present information on panicle length.

Panicle branches were longest with rough barnyardgrass and shortest with junglerice (Table 3.2), which reflects the large plant size of rough barnyardgrass. Rough barnyardgrass had a wide range of panicle branch lengths perhaps due to an admixture of varieties; var. *muricata* is reported to have shorter panicle branches than var. *microstachya* (Michael, 2003). However, panicle length did not correlate with seed production as rough barnyardgrass produced the largest and fewest seeds among the three species.

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Junglerice produced the highest amount of seed (539 seeds/panicle) and rough barnyardgrass produced the lowest (259 seeds/panicle) (Table 3.4). Accounting for the total number of panicles, the estimated total seed production per plant was significantly different among species. Junglerice produced the highest estimated number of seeds (72973 seeds/plant), banyardgrass had 31911 seeds/plant, and rough barnyardgrass produced an estimated 27589 seeds/plant. Coast cockspurgrass showed the lowest fecundity among the four species with only 25392 seeds/plant, but this is not definitive because this data was collected from only one accession.

The majority of junglerice (67%) and barnyardgrass (56%) had green hull color (Table 3.3). The majority of rough barnyardgrass (82%) had green-purple mixed hull coloration. Spikelet characteristics were among the most useful traits for identification of Echinochloa species. Junglerice spikelets were awnless (Table 3.3). Spikelets of barnyardgrass and rough barnyardgrass in Arkansas may or may not be awned and spikelets within a panicle may not all have awns. Further, the length of awns differed according to the position of spikelets within the panicle. About 56% of barnyardgrass accessions were awnless. Rough barnyardgrass occurs in two varieties: 1) var. *muricata* which is either awned or awnless and 2) var. *microstachya* which is generally awned. In this study, the majority (91%) of rough barnyardgrass spikelets had awns. Awn length was reported to be dependent on the relative humidity of its habitat (Maun and Barett, 1986). Thus, the awn length of rough barnyardgrass varied greatly, ranging from 1.8-9.48 mm while barnyardgrass had 1.5-16.7 mm awns (Table 3.2). With such a large variation in this trait, one can only associate the most obvious generalities to a species such as being predominantly awnless, predominantly long-awned or having long awns only on apical spikelets in a panicle.

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Spikelet characteristics (upper glume, lower glume, upper lemma, lower lemma, spikelet length and width) of the three species were significantly different (Table 3.5). Barnyardgrass had intermediate characteristics between junglerice and rough barnyardgrass, which creates potential for misidentification. Rough barnyardgrass had the longest (3.02-4.73 cm) and widest (1.24-2.43 cm) spikelets. There was a wide range of spikelet size among rough barnyardgrass accessions owing to the two varieties (var. *muricata* and var. *microstachya*) that differ significantly in these traits. Variety *muricata* was reported to have spikelets more than 3.5 mm long while var. *microstachya* have spikelets less than 3.5 mm long (Gould, 1972). Specific ranges were reported by Michael (2003): 3.5-5 mm for var. *muricata* and 2.8-3.5 mm for var. *microstachya*. In itself, abiding by this range is not helpful because of the large overlap in spikelet length between the two varieties. Junglerice had the smallest spikelets, 2.31-3.04 mm long and 1.11-1.44 mm wide. Spikelets of junglerice in Arkansas, USA were longer than what was reported in Milan, Italy which is less than 2.5 mm (Tabacchi et al., 2006).

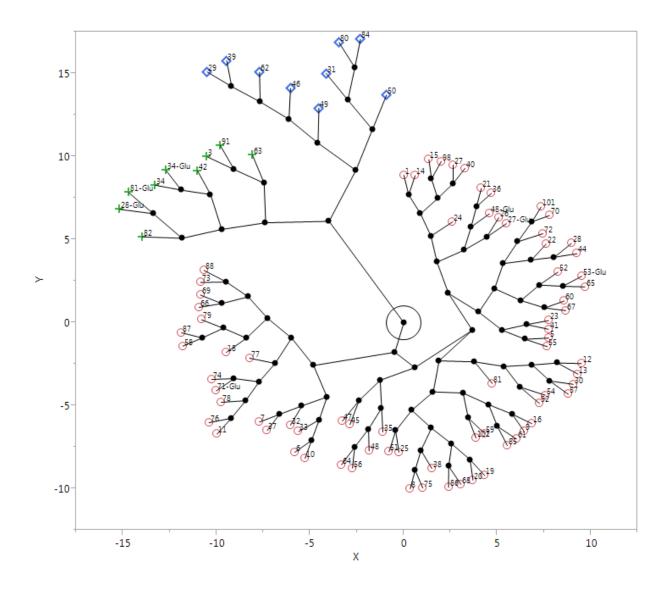


Figure 3.5 Castellation plot of Echinochloa in Arkansas, USA. Cluster 1 = junglerice (O); cluster 2 = barnyardgrass (+); cluster 3 = rough barnyardgrass (square). Cluster analysis included plant height at maturity, number of tillers, leaf length and width, panicle length, length of panicle branch, days to initiation of emergence, days to heading, days to maturity, awn length, spikelets length and width, length of upper lemma and lower lemma, length of upper glume and lower glume, panicle count, total seed per panicle and total seeds per plant.

Barnyardgrass spikelets were 2.95-3.80 mm long and 1.45-1.86 mm wide (Table 3.5).

These measurements overlap with the upper range of seed characteristics of junglerice and the lower range of measurements for rough barnyardgrass var. *microstachya*. Rough barnyardgrass had the longest upper glume (2.95-4.71 mm), lower glume (1.29-2.51 mm), upper lemma (2.54-4.23 mm) and lower lemma (2.94-4.71 mm) (Table 3.3). The barnyardgrass upper lemma (2.71-3.55 cm) and lower lemma (2.94-3.74 cm) were within the range of rough barnyardgrass; but the upper glumes (2.94-3.80 cm) and lower glumes (1.21-1.68 cm) were smaller than those of rough barnyardgrass. Junglerice had the shortest upper glume (2.23-2.98 mm), lower glume (0.98-1.44), upper lemma (1.91-2.75 mm) and lower lemma (2.15-2.96 mm). The majority of junglerice (73%) had brownish caryopsis color and the rest were a shade of green (Table 3.3). Barnyardgrass also had an equal proportion of brown and green caryopsis color.

Cluster analysis—Cluster analysis was conducted based on morphology (vegetative and reproductive traits) and phenology to verify the *Echinochloa* species identity (Figure 3.5). Cluster 1 was the largest cluster consisting of 73 accessions classified as junglerice, 1 accession classified as barnyardgrass (Acc. 13) and 1 accession of rough barnyardgrass (Acc. 81). Accessions in this cluster had short culms (80 cm), high tillering (70 tillers/plant), short (19.7 cm) and narrow flag leaf (1.3 cm), short panicles (13.6 cm), the shortest panicle branches (1-2.6 cm), no awn, the shortest spikelets (2.63 mm), the shortest upper (2.23-2.98 mm) and lower (0.98-1.59 mm) glumes, and the shortest upper (1.91-2.93 mm) and lower (2.15-2.96 mm) lemmas. The majority of accessions in this cluster had green leaf sheath (92%), light purple internodes, green leaf blade, green hull, horizontal leaf angle and open growth habit. The accessions in this cluster also were the earliest to exert 50 % of its panicles (38.5-58 DAE) and the earliest to mature. The barnyardgrass and rough barnyardgrass accessions in this cluster were awnless, had small spikelets and were morphologically similar to junglerice. The barnyardgrass in this cluster have longer panicle branches than junglerice. The rough barnyardgrass in this cluster also have longer panicles and panicle branches than junglerice. Cases like this beg for genetic verification of the 'rouge' species.

Cluster 2 consisted of eight accessions of barnyardgrass and 1 accession of rough barnyardgrass (Acc 81-G). Accessions in this cluster were the tallest plants (103 cm), but with intermediate flag leaf length (22.14 cm), flag leaf width (1.39 cm), panicle length (16.07 cm), awn length (3.57 mm), panicle branch length (3.57 cm), and spikelet characteristics relative to accessions in the other two clusters. The accessions initiated heading between 46-59 DAE and matured between 80 and 94 DAE. The majority of accessions had green leaf sheath, purple node, light purple internode, green leaf blade, green hull and open growth habit.

Cluster 3 exemplified rough banyardgrass. No other species in this cluster. All, except two, rough barnyardgrass accessions were placed in this cluster. Rough barnyardgrass accessions in this cluster were slightly shorter (98 cm) than the barnyardgrass accessions in Cluster 2 and were the latest to exert panicles (52.5-63 DAE) and mature (83-96 DAE). Rough barnyardgrass had the lowest tillering capacity (56 tillers/plant), longest (30.6 cm) and widest (1.56 cm) flag leaf, longest panicles (21.8 cm) and panicle branches (2.30-6.10 cm), longest awns (5.2 mm), longest (4.19 mm) and widest spikelets, longest upper (3.41- 4.71 mm) and lower (1.29-2.51 mm) glumes, and the longest upper (3.11- 4.23 mm) and lower (3.31 - 4.71 mm) lemma relative to plants in the other clusters. The majority of accessions had green leaf sheath (77.8%), purple node (44.44%), light purple internode, green leaf blade (77.78%), horizontal leaf angle and decumbent growth habit. All accessions had mixed purple-green hull color.

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Based on these data, the proposed identification keys for *Echinochloa* species (Figure 3.7) in Arkansas, USA are as follows:

Cluster 1

Cluster II

Cluster III

 Height at maturity 79.1-117.6 cm, flag leaf length more than 22 cm, flag leaf width 1.2-2.1 cm, panicle length 16.4-24.8 cm, awned, panicle branch 2.3-6.1 cm, spikelet length 3.5-4.7 mm, spikelet width 1.8-2.4 mm, length of upper glume 3.41-4.71 mm, length of lower glume 1.29-2.51 mm, length of upper lemma 3.11-4.23 mm, length of lower lemma 3.31-4.71 mm....*E. muricata*

Conclusion

The abundance of junglerice in Arkansas is under-reported in the literature. Species misidentification also resulted in the attribution of resistance among junglerice populations to barnyardgrass. The phenological and morphological traits vary widely within *Echinochloa* species; junglerice is the least variable. Junglerice is awnless, short, with high tillering capacity, flowers and matures early, and produces the highest number of seeds. Characteristics of spikelets, panicles and leaves are the most reliable traits to identify the species in the field. Closer inspection of panicles and spikelet in the laboratory, using a dissecting microscope is necessary to verify the identity of plants showing intermediate characteristics. Since *Echinochloa* species tend to intergrade, follow-up experiments using molecular tools are needed to develop species-specific molecular markers to verify species identity. Diversity in plant traits may also result in differential response to some herbicides, different rates and patterns of resistance evolution, and differential adaptation to cultural production practices.

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Accessions	Year collected	County	Latitude	Longitude
1	2010	Jefferson	34°21′46″ N	91°55′25.26″ W
3	2010	Ashley	33°12′18″ N	91°31′24.66″ W
4	2010	Chicot		
5	2010	Chicot	33°26′01″ N	91°27′10.62″ W
6	2010	Lincoln	33°55′20″ N	91°39′39.84″ W
7	2010	Monroe	34°40′14″ N	91°14′58.32″ W
8	2010	Arkansas	34°05′09″ N	91°26′24.24″ W
9	2010	Arkansas	34°09′39″ N	91°20′40.2″ W
10	2010	Desha	33°54′14″ N	91°25′10.74″ W
11	2010	Arkansas	34°23′29″ N	91°15′47.82″ W
12	2010	Monroe	34°42′32″ N	91°08′05.04″ W
13	2010	Prairie	34°44′52″ N	91°21′42.78″ W
14	2010	Prairie	34°58′40″ N	91°31′57.06″ W
15	2010	Monroe	34°55′39″ N	91°13′14.22″ W
16	2010	Lee	34°49′02″ N	90°54′54″ W
18	2010	Lonoke	34°45′44″ N	91°51′12.3″ W
19	2010	Prairie	34°48′06″ N	91°29′28.08″ W
20	2010	Prairie	34°47′19″ N	91°36′36″ W
21	2010	Monroe	34°44′42″ N	91°08′49.08″ W
22	2010	Cross	35°16′19″ N	90°36′56.88″ W
23	2010	Crittenden	35°22′34″ N	90°21′33.84″ W
24	2010	Ashley	33°14′42″ N	91°27′46.44″ W
25	2010	Chicot	33°29′26″ N	91°24′12.72″ W
26	2010	Chicot	33°21′21″ N	91°26′04.74″ W
27	2010	Prairie		
27-G ^a	2010	Prairie		
28	2010	Lincoln	33°58′36″ N	91°38′59.7″ W
28-G ^a	2010	Lincoln	33°58′36″ N	91°38′59.7″ W
29	2010	Lawrence	36°08′27″ N	91°00′20.34″ W
30	2010	Lawrence	35°56′01″ N	90°56′23.1″ W
31	2010	Lawrence	35°56′28″ N	90°56′33.84″ W
32	2010	Lawrence	36°04′31″ N	90°58′38.7″ W
33	2010	Lawrence	36°00′48″ N	90°54′18.48″ W
34	2010	Lawrence	36°07′48″ N	90°54′10.68″ W

Table 3.1. Accessions collected from different counties across Arkansas, USA in 2010 and 2011.

^a Accession with reduced sensitivity to glufosinate herbicide

Accessions	Year collected	County	Latitude	Longitude
34-G ^a	2010	Lawrence	36°07′48″ N	90°54′10.68″ W
35	2010	Monroe	34°48′04″ N	91°06′49.38″ W
36	2010	Monroe	34°46′49″ N	91°14′39.9″ W
37	2010	Lonoke	34°50′56″ N	91°45′59.28″ W
38	2010	Cross	35°19′24″ N	90°56′53.28″ W
39	2010	Mississippi	35°13′27″ N	90°48′42.48″ W
40	2010	Poinsett	35°39′50″ N	90°17′39.66″ W
41	2010	Mississippi	35°48′26″ N	90°04′20.82″ W
42	2010	Mississippi	35°36′41″ N	90°05′21.72″ W
44	2010	Mississippi	35°56′04″ N	90°01′06.66″ W
45	2010	Lawrence	35°55′28″ N	91°09′20.76″ W
46	2010	Clay	36°23′39″ N	90°23′23.22″ W
47	2010	Lonoke	34°51′14″ N	91°52′30.48″ W
48	2010	Jackson	35°51′33″ N	91°08′59.4″ W
48-G ^a	2010	Jackson	35°51′33″ N	91°08′59.4″ W
49	2010	Greene	35°59′39″ N	90°28′23.7″ W
50	2010	Greene	35°59′14″ N	90°29′38.52″ W
51	2010	Greene	36°09′26″ N	90°17′34.14″ W
52	2010	Greene	36°07′53″ N	90°18′22.98″ W
53-G ^a	2010	Greene	36°04′09″ N	90°44′03.18″ W
54	2010	Greene	36°03′16″ N	90°45′37.38″ W
55	2010	Prairie	34°55′59″ N	91°22′53.76″ W
56	2010	Prairie	34°46′14″ N	91°40′33.6″ W
56-G ^a	2010	Prairie	34°46′14″ N	91°40′33.6″ W
57	2010	Prairie	35°01′01″ N	91°22′26.46″ W
58	2010	Lincoln	34°00′25″ N	91°41′28.32″ W
59	2010	Prairie	34°44′04″ N	91°37′07.26″ W
60	2010	Prairie	35°01′23″ N	91°22′53.76″ W
61	2010	St. Francis	34°57′39″ N	90°40′21.42″ W
62	2010	St. Francis	34°58′54″ N	90°48′37.2″ W
63	2010	White	35°07′23″ N	91°41′40.56″ W
64	2010	White	35°09′02″ N	91°37′55.14″ W
65	2010	White	35°07′16″ N	91°35′47.58″ W
66	2010	White	35°05′29″ N	91°37′10.56″ W

Table 3.1. Accessions collected from different counties across Arkansas, USA in 2010 and 2011. (cont.)

^a Accession with reduced sensitivity to glufosinate herbicide

Accessions	Year collected	County	Latitude	Longitude
67	2010	Woodruff	35°19′54″ N	91°24′24.9″ W
68	2010	White	35°22′02″ N	91°28′33.12″ W
69	2010	Phillips	34°32′59″ N	90°49′18.36″ W
70	2010	Phillips	34°26′09″ N	90°37′50.58″ W
71-G ^a	2010	Phillips	34°30′08″ N	90°46′30.6″ W
72	2010	Phillips	34°28′04″ N	90°49′34.32″ W
73	2010	Phillips	34°25′32″ N	90°38′22.8″ W
74	2010	Clay	36°25′34″ N	90°36′50.28″ W
75	2010	Phillips	34°28′26″ N	90°43′59.94″ W
76	2010	Drew	33°39′53″ N	91°30′41.28″ W
77	2010	Drew	33°39′17″ N	91°30′57.54″ W
78	2010	Drew	33°42′36″ N	91°31′16.62″ W
79	2010	Poinsett	35°38′33″ N	90°49′58.5″ W
80	2010	Poinsett	35°32′49″ N	91°00′22.68″ W
81	2010	Jackson	35°31′49″ N	91°10′24.06″ W
81-G ^a	2010	Jackson	35°31′49″ N	91°10′24.06″ W
82	2010	Jackson	35°37′21″ N	91°07′09.3″ W
84	2010	Jackson	35°31′44″ N	91°10′34.92″ W
85	2011	Prairie		
86	2011	Prairie		
87	2011	Prairie		
88	2011	Prairie		
91	2011	Monroe		
92	2011	Jackson		
98	2011	Lawrence		
101	2011	Prairie		
102	2011	Prairie		

Table 3.1. Accessions collected from different counties across Arkansas, USA in 2010 and 2011. (cont.)

^a Accession with reduced sensitivity to glufosinate herbicide

Table 3.2. Quantitative morphological traits of *Echinochloa* species, grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013.

	Heigh	Height at maturity			Number of tillers			Leaf length		Leaf width			Panicle length		th	Awn width ^a		
Species ^a	(cm)						(cm)			(cm)			(cm)			(mm)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
junglerice	79.8	65.4	94.3	70	31	129	19.7	13.4	24.6	1.3	1.0	1.5	13.5	10.5	19.2	-	-	-
rough barnyardgrass	97.2	79.1	117.6	57	40	65	28.7	19.8	42.0	1.5	1.2	2.1	21.5	16.4	24.8	5.20	1.80	9.48
barnyardgrass	101.9	87.3	125.5	63	20	111	22.0	16.2	30.0	1.4	1.2	1.5	15.7	13.8	20.8	6.74	1.00	16.7
coast cockspurgrass	81.1	53.3	108.9	50	37	63	17.5	16.3	18.8	1.2	1.1	1.4	12.6	11.5	13.8	21.06	18.21	23.9
LSD ^b	4.5			10.4			3.1			0.11			1.8			1.09		

^aAwns were present only on barnyardgrass, rough barnyardgrass, and coast cockspurgrass. Mean, minimum and maximum values were calculated only from accessions with awns. ^b Means were compared using Fisher's LSD test (P=0.05). Data were averaged across accessions and four replications per accession.

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Table 3.2. Quantitative morphological traits of *Echinochloa* species, grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

	Par	nicle co	unt	Leng	th of pa	nicle	Days to	o initiat	ion of	Days	s to hea	ding	Days to maturity		
Species				bı	ranch(cr	n)	emerg	gence (I	DAP)		(DAE)			(DAE)	
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
junglerice	140	63	258	1.8	1.0	2.6	5.0	3.0	8.0	49	39	58	82	73	94
rough barnyardgrass	56	40	65	3.6	2.3	6.8	6.0	4.0	8.0	56	42	63	92	84	96
barnyardgrass	63	20	111	3.2	2.2	3.8	5.0	2.0	8.0	49	45	59	86	80	94
coast cockspurgrass	50			2.0	1.96	2.04	6.8	6.6	7	59	55	62	95	92	98
LSD ^a	12			0.5			0.7			3.6			3.5		

^a Means were compared using Fisher's LSD test (P=0.05). Data were averaged across accessions and four replications per accession.

		Fre	equency distribu	ition
Characteristics	Category	junglerice	barnyard-	rough
Characteristics	Culogory			barnyardgrass
	Brownish	73	44	45
Caryopsis color	Greenish	27	44	45
	Yellowish	0	11	9
Leaf sheath color	Green	92	89	82
Leaf sileatil color	Light green	1	11	0
	Green-purple mixture	7	0	18
	Green	18	22	18
	Light green	33	11	27
Node color	Purple	37	67	37
	Green-purple mixture	1	0	18
	Light Purple	11	0	0
	Green	8	0	9
	Light green	5	11	0
Internode color	Purple	11	11	27
	Purple-green mixture	45	67	55
	Light purple	30	11	9
	Green	93	89	82
Leaf blade color	Light green	7	11	0
Leaf blade color	Green with purple			
	margin	0	0	18
	Erect	30	22	9
Leaf Angle	Horizontal	49	67	55
Leai Aligie	Drooping	3	11	36
	45 angle	18	0	0
	Open	52	56	27
Growth habit	Prostrate	1	0	0
	Decumbent	47	44	73
Hull color at	Green	67	56	18
maturity	Purple	4	0	0
	Green-purple mixture	29	44	82
Awn	Awn	0	56	91
	Awnless	100	44	9

Table 3.3. Selected qualitative traits of *Echinochloa* species grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013.

		Total seeds/p	oanicle		Total seeds/p	olant
Species	Mean	Min	Max	Mean	Min	Max
junglerice	539	234	1739	72973	9098	217217
barnyardgrass	345	249	396	31911	7186	71494
rough barnyardgrass	259	107	565	27589	13189	37589
coast cockspurgrass	510			25392		
LSD ^b	156			25220		

Table 3.4. Seed production of *Echinochloa* species averaged across accessions.

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^a Data were obtained from 94 accessions with four biological replicates per accession. ^b Means were compared using Fisher's LSD test (P=0.05). *E. walteri* was excluded from the analysis because only one accession represented this specie.

	Upp	er glu	me	Low	er glu	me	Uppe	er lem	ma	Low	er lem	ma	Spike	elet ler	igth	Spike	elet wi	dth
Species ^a		(mm)		((mm)			(mm)		((mm)		((mm)		(mm)	
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
junglerice	2.57	2.23	2.98	1.21	0.98	1.44	2.33	1.91	2.75	2.54	2.15	2.96	2.62	2.31	3.04	1.27	1.11	1.44
barnyardgrass	3.34	2.94	3.80	1.42	1.21	1.68	3.14	2.71	3.55	3.31	2.94	3.74	3.38	2.95	3.80	1.62	1.45	1.86
rough barnyardgrass	3.97	2.95	4.71	1.65	1.29	2.51	3.46	2.54	4.23	3.91	2.94	4.71	4.01	3.02	4.73	1.95	1.24	2.43
coast cockspurgrass	2.55 ss		1.21			2.31			2.51		2.58			1.27				
LSD ^b	0.23			0.14			0.22			0.23			0.22			0.13		

Table 3.5. Quantitative, seed characteristics of *Echinochloa* species, averaged across accessions.

ΓΓ

^a Data were obtained from 94 accessions with four biological replicates per accession, and 2 florets per biological replicate were measured. ^b Means were compared using Fisher's LSD test (P=0.05). *E. walteri* was excluded from the analysis because only one accession represented this specie.

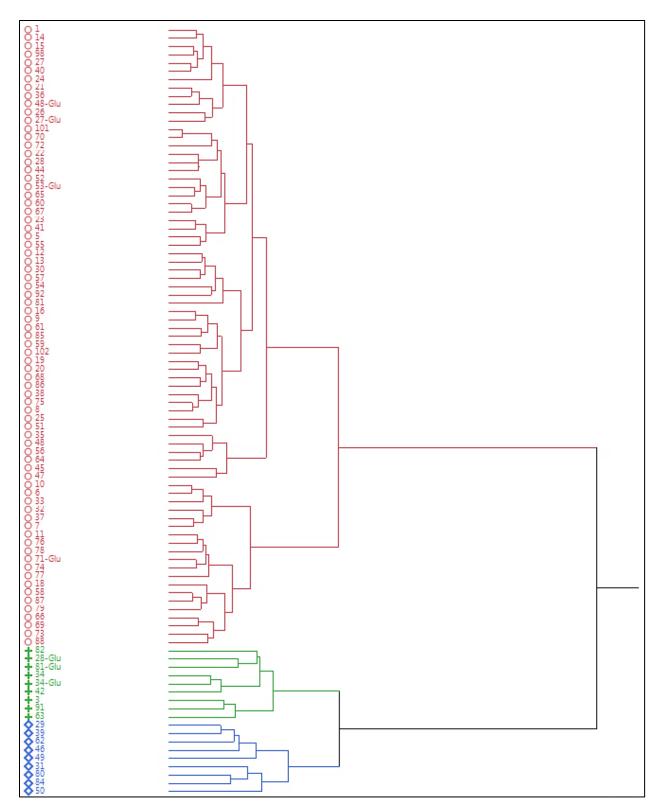


Figure 3.6 Dendogram of *Echinochloa* species from Arkansas, USA based on 10 vegetative and 9 reproductive traits.



Figure 3.7 Pictures showing panicle, lower glume, upper lemma, lower lemma and upper glume of *Echinochloa* species from Arkansas, USA.



Figure 3.8 Various growth habits (decumbent, open, prostrate) of *Echinochloa* species from Arkansas, USA.

рН	Р	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	В	NO ₃ - N+NO ₂ - N	NH4-N	Sand	Silt	Clay	CEC
					m	g/kg									%		cmol ⁺ /Kg
5.56	83	103	902	33	12	10	229	42.3	1.3	0.8	0.1	0.87	24.87	25	63	12	9

Appendix 3.1 Soil test result 2013 (Mehlich 3, soluble salts and specific ion electrode method)

Appendix 3.2 Soil test result 2012 (Mehlich 3, soluble salts and specific ion electrode method)

pН	Р	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	В	NO3- N+NO2- N		Sand	Silt	Clay	CEC
					mg	/Kg									%		cmol ⁺ /Kg
6.1	66	87	1281	82	16	14	330	51	1.62	1.13	0.25	32.8	11.9	18	70.4	11.6	11

Acc. number	Species	Height at maturity (cm)	Number of tillers	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Awn length (mm)	Length of panicle branch (cm)	Days to emergence (DAP)	Days to heading (DAE)	Days to maturity (DAE)
1	junglerice	79.3	82	22.1	1.4	14.5	0	1.6	5	56	83
3	barnyardgrass	90.8	41	25.7	1.5	14.0	0	2.9	6	54	91
4	coast cockspurgrass	81.1	50	17.5	1.2	12.6	21.00	2.0	7	59	95
5	junglerice	77.3	84	17.0	1.1	17.4	0	1.8	4	56	86
6	junglerice	85.6	79	19.7	1.4	11.2	0	1.6	4	52	79
7	junglerice	86.8	78	19.1	1.4	14.6	0	1.5	5	49	79
8	junglerice	82.8	68	19.5	1.3	13.6	0	1.9	4	45	79
9	junglerice	77.0	46	20.7	1.2	11.2	0	1.9	4	54	81
10	junglerice	85.9	85	24.0	1.4	10.9	0	1.6	4	54	76
11	junglerice	74.5	56	19.2	1.3	13.7	0	1.4	5	40	73
12	junglerice	82.0	56	20.4	1.3	13.1	0	1.1	5	51	87
13	barnyardgrass	87.3	65	19.8	1.3	14.9	0	2.2	5	45	87
14	junglerice	85.2	88	21.5	1.4	14.7	0	1.8	5	53	81

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013.

Acc.	Species	Height at	Number	Leaf	Leaf	Panicle	Awn	Length of	Days to	Days to	Days to
number		maturity	of tillers	length	width	length	length	panicle	emergence	heading	maturity
		(cm)		(cm)	(cm)	(cm)	(mm)	branch	(DAP)	(DAE)	(DAE)
								(cm)			
15	junglerice	79.5	80	19.8	1.3	14.2	0	2.4	5	55	88
16	junglerice	78.0	59	20.2	1.3	13.9	0	1.8	5	51	80
18	junglerice	82.5	57	22.5	1.4	14.4	0	1.5	5	48	73
19	junglerice	70.2	61	22.5	1.3	13.4	0	1.7	5	45	87
20	junglerice	74.3	77	19.9	1.4	14.3	0	1.9	5	48	87
21	junglerice	77.1	64	19.6	1.3	13.0	0	1.9	5	54	88
22	junglerice	88.3	79	24.6	1.5	15.9	0	1.8	4	52	84
23	junglerice	68.2	84	17.4	1.2	14.2	0	1.6	5	44	83
24	junglerice	75.3	92	18.6	1.4	14.0	0	2.2	4	55	86
25	junglerice	77.4	53	15.5	1.1	11.7	0	1.5	5	49	87
26	junglerice	79.5	56	17.9	1.3	11.7	0	1.4	4	54	94
27	junglerice	84.0	70	21.5	1.3	15.3	0	1.9	4	56	85

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc. number	Species	Height at maturity (cm)	Number of tillers	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Awn length (mm)	Length of panicle branch (cm)	Days to emergence (DAP)	Days to heading (DAE)	Days to maturity (DAE)
28	junglerice	80.9	69	17.9	1.4	16.9	0	1.4	5	52	88
29	rough barnyardgrass	105.9	54	30.2	1.6	22.6	2	2.9	6	62	90
30	junglerice	88.7	57	17.6	1.4	15.2	0	1.7	5	51	85
31	rough barnyardgrass	87.7	63	42	1.2	16.4	9	2.4	5	61	95
32	junglerice	83.9	81	17.3	1.3	12.3	0	2.2	6	53	74
33	junglerice	81.4	87	18.2	1.3	12.8	0	1.3	4	51	73
34	barnyardgrass	106.4	78	20.7	1.4	17.4	5	3.8	5	47	80
35	junglerice	67.6	59	17.5	1.2	12.2	0	1.9	8	50	81
36	junglerice	79.7	77	20.3	1.3	12.6	0	2.5	4	53	86
37	junglerice	78.0	71	19.2	1.3	13.6	0	1.5	6	52	77
38	junglerice	79.6	54	20.0	1.2	14.2	0	1.0	5	46	81
39	rough barnyardgrass	91.8	56	23.3	1.4	22.5	5	2.8	6	55	89
40	junglerice	77.2	75	20.6	1.3	12.3	0	2.2	4	55	89

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc. number	Species	Height at maturity (cm)	Number of tillers	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Awn length (mm)	Length of panicle branch (cm)	Days to emergence (DAP)	Days to heading (DAE)	Days to maturity (DAE)
41	junglerice	72.3	77	16.3	1.2	17.1	0	1.8	5	49	87
42	barnyardgrass	104.9	111	19.3	1.2	14.7	0	3.0	5	47	86
44	junglerice	81.0	61	20.9	1.3	14.2	0	1.8	5	53	86
45	junglerice	81.5	92	20.3	1.5	12.6	0	1.7	8	57	86
46	rough barnyardgrass	92.2	60	22.0	1.4	23.7	6	6.1	7	55	94
47	junglerice	76.3	129	23.3	1.3	12.3	0	2.0	7	47	87
48	junglerice	77.0	80	19.8	1.3	12.4	0	2.0	7	52	92
49	rough barnyardgrass	79.1	64	22.1	1.6	23	8.48	2.3	4	60	84
50	rough barnyardgrass	117.6	52	39.7	2.1	24.8	1.8	4.1	4	54	90
51	junglerice	65.4	63	13.4	1.3	12.3	0	1.7	4	43	86
52	junglerice	82.2	81	17.7	1.3	13.2	0	1.8	5	40	84
54	junglerice	83.9	61	21.6	1.3	19.2	0	1.8	5.8	53	83
55	junglerice	73.6	97	18.7	1.2	12.6	0	1.2	4.8	52	85

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc.	Species	Height at	Number	Leaf	Leaf	Panicle	Awn	Length of	Days to	Days to	Days to
number		maturity	of tillers	length	width	length	length	panicle	emergence	heading	maturity
		(cm)		(cm)	(cm)	(cm)	(mm)	branch	(DAP)	(DAE)	(DAE)
			= 2	10.1				(cm)			0.1
56	junglerice	72.5	73	18.1	1.2	11.2	0	1.4	6	50	91
57	junglerice	74.9	51	19.9	1.2	12.3	0	1.8	4	47	81
58	junglerice	82.3	61	23.3	1.4	13.6	0	2.1	4	46	80
59	junglerice	94.3	58	22.7	1.2	14.2	0	1.9	4	53	82
60	junglerice	80.0	75	20.0	1.4	16.0	0	1.6	5	45	81
61	junglerice	85.1	45	18	1.3	12.6	0	1.7	6	49	84
62	rough barnyardgrass	101.2	40	34.2	1.5	22.4	3	2.4	8	53	93
63	barnyardgrass	125.5	20	19.9	1.4	15.9	0	3.2	8	48	89
64	junglerice	83.7	75	19.2	1.3	13.4	0	1.9	6	55	88
65	junglerice	83.0	75	18.2	1.4	13.2	0	2.3	6	48	87
66	junglerice	84.0	77	19.0	1.4	12.6	0	1.7	6	39	77

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc. number	Species	Height at maturity (cm)	Number of tillers	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Awn length (mm)	Length of panicle branch	Days to emergence (DAP)	Days to heading (DAE)	Days to maturity (DAE)
		(cm)		(em)	(CIII)	(em)	(IIIII)	(cm)		(DAL)	(DAL)
67	junglerice	82.3	89	18.9	1.3	18.0	0	1.9	5	46	80
68	junglerice	75.5	76	21.8	1.2	11.9	0	1.8	5	53	80
69	junglerice	76.0	93	19.9	1.3	10.8	0	1.5	5	39	77
70	junglerice	84.3	81	22.1	1.2	12.4	0	1.8	5	48	84
72	junglerice	85.4	88	18.2	1.4	12.8	0	1.5	5	50	80
73	junglerice	74.3	65	16.9	1.1	10.5	0	1.7	6	41	75
74	junglerice	74.7	73	15.7	1.2	10.5	0	2.1	5	41	74
75	junglerice	77.7	58	17.6	1.3	13.6	0	2.2	5	49	81
76	junglerice	75.7	54	17.8	1.2	10.6	0	1.7	4	41	75
77	junglerice	79.4	57	17.7	1.3	14.3	0	2.0	5	45	75
78	junglerice	76.1	57	16.9	1.0	11.1	0	1.9	4	43	74

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc. number	Species	Height at maturity (cm)	Number of tillers	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Awn length (mm)	Length of panicle branch (cm)	Days to emergence (DAP)	Days to heading (DAE)	Days to maturity (DAE)
79	junglerice	78.8	42	22.4	1.3	12.5	0	1.6	5	42	79
80	rough barnyardgrass	96.0	65	30.1	1.6	17.6	8	4.6	6	60	96
81	rough barnyardgrass	87.9	62	19.8	1.2	21.7	0	2.6	5	42	91
82	barnyardgrass	90.4	63	21.8	1.4	15.1	17	3.7	4	47	85
84	rough barnyardgrass	110.4	52	31.4	1.7	23.0	6	3.1	8	63	94
85	junglerice	79.0	53	18.8	1.4	13.1	0	1.6	5	54	84
86	junglerice	81.8	66	21.4	1.3	14.9	0	1.5	6	50	84
87	junglerice	76.1	51	21.6	1.4	13.8	0	2.1	5	41	76
88	junglerice	76.7	78	19.5	1.3	13.7	0	2.2	6	42	76
91	barnyardgrass	108.8	62	24.7	1.5	20.8	0	1.8	5	59	85
92	junglerice	91.0	31	22.9	1.3	16.5	0	1.8	6	49	91
98	junglerice	83.4	75	20.3	1.3	12.0	0	2.0	4	52	85

Appendix 3.3 Quantitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Appendix 3.3 Quantitative morphological traits of <i>Echinochloa</i> accessions grown in a common garden at the Main Experiment
Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc.	Species	Height at	Number	Leaf	Leaf	Panicle	Awn	Length of	Days to	Days to	Days to
number		maturity	of tillers	length	width	length	length	panicle	emergence	heading	maturity
		(cm)		(cm)	(cm)	(cm)	(mm)	branch	(DAP)	(DAE)	(DAE)
								(cm)			
101	junglerice	88.1	78	22.7	1.2	13.9	0	1.7	5	50	87
102	junglerice	86.8	49	19.5	1.2	17.0	0	1.7	3	48	87
27-Glu	junglerice	85.2	60	23.4	1.4	14.8	0	2.0	3	56	91
28-Glu	barnyardgrass	103.9	50	30.0	1.5	14.5	1	3.3	2	53	94
34-Glu	barnyardgrass	99.1	76	16.2	1.2	13.8	4	3.6	4	46	82
48-Glu	junglerice	76.1	51	18.9	1.4	12.4	0	2.1	6	58	90
53-Glu	junglerice	83.4	85	22.4	1.4	16.6	0	2.6	5	47	84
71-Glu	junglerice	73.3	56	18.9	1.3	11.0	0	2.3	4	39	73
81-Glu	rough barnyardgrass	99.2	54	21.0	1.4	18.4	5	6.8	4	55	93

Appendix 3.4 Qualitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013.

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
1	junglerice	Jefferson	Brown	Green	Light green	Light <u>p</u> urple	Green	Green- purple mixture	Horizontal	Open
3	barnyardgrass	Ashley	Brown	Green	Green	Light green	Green	Green	Drooping	Decumbent
4	coast cockspurgrass	Chicot	Green	Green	Green	Light purple	Green	Green- purple mixture	Drooping	Decumbent
5	junglerice	Chicot	Brown	Green	Light green	Light purple	Green	Green	Erect	Decumbent
6	junglerice	Lincoln	Brown	Green	Light green	Green- purple mixture	Green	Green	Horizontal	Decumbent
7	junglerice	Monroe	Green	Green	Light green	Green	Green	Green	Erect	Open
8	junglerice	Arkansas	Brown	Green	Light green	Light purple	Green	Green	45 °	Open
9	junglerice	Arkansas	Brown	Green	Green	Green- purple mixture	Green	Green	Horizontal	Decumbent
10	junglerice	Desha	Brown	Green	Light green	Green- purple mixture	Green	Purple	Horizontal	Decumbent

Appendix 3.4 Qualitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
11	junglerice	Arkansas	Brown	Green	Light purple	Green- purple mixture	Green	Green	Drooping	Decumbent
12	junglerice	Monroe	Brown	Green	Green	Light purple	Green	Green	Erect	Decumbent
13	barnyardgrass	Prairie	Green	Green	Purple	Green- purple mixture	Green	Green- purple mixture	Horizontal	Decumbent
14	junglerice	Prairie	Brown	Green	Light purple	Light purple	Green	Green- purple mixture	Horizontal	Decumbent
15	junglerice	Monroe	Brown	Green	Light green	Light purple	Green	Green	45 °	Decumbent
16	junglerice	Lee	Green	Green	Green	Green- purple mixture	Green	Green- purple mixture	45 °	Decumbent
18	junglerice	Lonoke	Brown	Green	Green- purple mixture	Light purple	Green	Green- purple mixture	Horizontal	Decumbent
19	junglerice	Prairie	Green	Green	Green	Green- purple mixture	Green	Green- purple mixture	45 °	Open

Appendix 3.4 Qualitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
20	junglerice	Prairie	Brown	Green	Purple	Green- purple mixture	Green	Green	45 °	Decumbent
21	junglerice	Monroe	Green	Green	Green	Green- purple mixture	Green	Green	Erect	Open
22	junglerice	Cross	Brown	Green	Purple	Light purple	Green	Green	Erect	Open
23	junglerice	Crittenden	Brown	Green	Purple	Light purple	Green	Green- purple mixture	Erect	Open
24	junglerice	Ashley	Brown	Green	Green	Green	Green	Green	Horizontal	Open
25	junglerice	Chicot	Brown	Green	Light green	Green- purple mixture	Green	Green	Horizontal	Open
26	junglerice	Chicot	Green	Green	Purple	Purple	Green	Green- purple mixture	Horizontal	Decumbent
27	junglerice	Prairie	Brown	Green	Purple	Purple	Green	Green- purple mixture	Horizontal	Decumbent
28	junglerice	Lincoln	Brown	Green	Purple	Light purple	Green	Green	Horizontal	Decumbent

Appendix 3.4 Qualitative morphological traits of *Echinochloa* accessions grown in a common garden at the Main Experiment Station, Fayetteville, AR, USA in 2012 and 2013 (cont.).

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.		-	color	sheath	color	node	blade	color at	Angle	habit
				color		color		maturity		
29	rough barnyardgrass	Lawrence	Brown	Green	Purple	Light purple	Green	Green- purple mixture	Horizontal	Decumbent
30	junglerice	Lawrence	Green	Green	Light green	Light purple	Green	Green- purple mixture	Erect	Decumbent
31	rough barnyardgrass	Lawrence	Green	Green	Green	Purple	Green	Green- purple mixture	Horizontal	Decumbent
32	junglerice	Lawrence	Brown	Green	Purple	Light purple	Green	Green	Horizontal	Open
33	junglerice	Lawrence	Brown	Green	Light green	Green	Green	Green	Horizontal	Open
34	barnyardgrass	Lawrence	Brown	Green	Purple	Green- purple mixture	Green	Green- purple mixture	Horizontal	Decumbent
35	junglerice	Monroe	Green	Green	Light green	Light purple	Green	Green	Horizontal	Open
36	junglerice	Monroe	Green	Green	Light green	Green- purple mixture	Green	Green	Erect	Open
37	junglerice	Lonoke	Brown	Green	Light green	Green	Green	Green	Erect	Decumbent

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath	color	node	blade	color at	Angle	habit
				color		color		maturity		
38	junglerice	Cross	Brown	Green	Light green	Light green	Green	Green	Erect	Open
39	rough barnyardgrass	Mississippi	Green	Green- purple mixture	Green- purple mixture	Green- purple mixture	Green with purple Margin	Green- purple mixture	Drooping	Open
40	junglerice	Poinsett	Brown	Green	Purple	Light purple	Green	Green- purple mixture	Horizontal	Decumbent
41	junglerice	Mississippi	Brown	Green	Purple	Green- purple mixture	Green	Green	Erect	Open
42	barnyardgrass	Mississippi	Green	Green	Purple	Green- purple mixture	Green	Green	Erect	Open
44	junglerice	Mississippi	Brown	Green	Purple	Purple	Green	Green	Erect	Open
45	junglerice	Lawrence	Brown	Green- purple mixture	Purple	Purple	Green	Green- purple mixture	Horizontal	Decumbent
46	rough barnyardgrass	Clay	Green	Green	Purple	Purple	Green	Green- purple mixture	Horizontal	Decumbent
47	junglerice	Jackson	Green	Green	Light green	Light green	Green	Green	Horizontal	Open

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
48	junglerice	Jackson	Brown	Green	Light green	Light green	Green	Green	Horizontal	Open
49	rough barnyardgrass	Greene	Brown	Green	Purple	Green- purple mixture	Green	Green- purple mixture	Horizontal	Decumbent
50	rough barnyardgrass	Greene	Green	Green	Light green	Green- purple mixture	Green	Green- purple mixture	Drooping	Open
51	junglerice	Greene	Green	Green	Purple	Light purple	Green	Green	Horizontal	Decumbent
52	junglerice	Greene	Brown	Green	Light green	Green- purple mixture	Green	Green	Horizontal	Open
54	junglerice	Greene	Brown	Green	Light green	Purple	Green	Purple	Drooping	Decumbent
55	junglerice	Prairie	Brown	Green	Green	Green- purple mixture	Green	Green	Erect	Decumbent
56	junglerice	Prairie	Brown	Green	Purple	Green- purple mixture	Green	Green	45 °	Open

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
57	junglerice	Prairie	Brown	Green	Purple	Light purple	Green	Green- purple mixture	Erect	Decumbent
58	junglerice	Lincoln	Brown	Green	Light green	Green- purple mixture	Green	Green	Erect	Open
59	junglerice	Prairie	Brown	Green	Green	Green- purple mixture	Green	Green- purple mixture	Horizontal	Open
60	junglerice	Prairie	Green	Green	Green	Green- purple mixture	Green	Green	Erect	Decumbent
61	junglerice	St. Francis	Brown	Green	Light green	Green- purple mixture	Green	Green	Horizontal	Open
62	rough barnyardgrass	St. Francis	Brown	Green	Light green	Green- purple mixture	Green	Green- purple mixture	Drooping	Decumbent
63	barnyardgrass	White	Green	Green	Purple	Purple	Green	Green	Horizontal	Open
64	junglerice	White	Brown	Green	Light green	Light purple	Green	Green- purple mixture	45 °	Open

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
65	junglerice	White	Green	Green	Purple	Light purple	Green	Green	Horizontal	Open
66	junglerice	White	Green	Green	Light purple	Green- purple mixture	Green	Green	Horizontal	decumbent
67	junglerice	Woodruff	Brown	Green	Green	Green- purple mixture	Green	Green	Erect	Open
68	junglerice	White	Brown	Green	Light purple	Green- purple mixture	Purple	Green	Horizontal	Open
69	junglerice	Phillips	Green	Green- purple mixture	Purple	Green- purple mixture	Green	Green- purple mixture	Horizontal	decumbent
70	junglerice	Phillips	Brown	Green- purple mixture	Purple	Purple	Green	Green	Horizontal	decumbent
72	junglerice	Phillips	Brown	Green- purple mixture	Green	Green- purple mixture	Green	Green- purple mixture	Horizontal	decumbent
73	junglerice	Phillips	Green	Green	Purple	Light purple	Light green	Purple	Horizontal	Open

Acc. no.	Species	County	Caryopsis color	Leaf sheath color	Node color	Inter- node color	Leaf blade	Hull color at maturity	Leaf Angle	Growth habit
74	junglerice	Clay	Brown	Green	Purple	Green- purple mixture	Green	Green- purple mixture	45 °	Decumbent
75	junglerice	Phillips	Brown	Green	Light green	Green	Green	Green	45 °	Open
76	junglerice	Drew	Brown	Green	Light purple	Green- purple mixture	Green with purple Margin	Green	45 °	Open
77	junglerice	Drew	Brown	Green	Green	Light green	Green with purple Margin	Green	45 °	Open
78	junglerice	Drew	Brown	Green	Light purple	Light purple	Green	Green- purple mixture	45 °	Decumbent
79	junglerice	Poinsett	Green	Green	Purple	Green- purple mixture	Green	Green	Horizontal	Prostrate
80	rough barnyardgrass	Poinsett	Green	Green- purple mixture	Green- purple mixture	Green- purple mixture	Green with purple Margin	Green- purple mixture	Horizontal	Open

Appendix 3.4 Qualitative morphological traits of Echinochloa accessions grown in a common garden at the Main Experiment Station,	
Fayetteville, AR, USA in 2012 and 2013 (cont.).	

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
81	rough barnyardgrass	Jackson	Yellow	Green	Light green	Green	Green	Green	Erect	Decumbent
82	barnyardgrass	Jackson	Brown	Green	Purple	Green- purple mixture	Green	Green	Erect	Open
84	rough barnyardgrass	Jackson	Brown	Green	Purple	Purple	Green	Green- purple mixture	Drooping	Decumbent
85	junglerice	Prairie	Brown	Light green	Purple	Green- purple mixture	Green with purple Margin	Green- purple mixture	Horizontal	Open
86	junglerice	Prairie	Brown	Green	Light purple	Green- purple mixture	Green	Green	45 °	Open
87	junglerice	Prairie	Green	Green	Light purple	Light purple	Green	Green	Erect	Open
88	junglerice	Prairie	Green	Green	Light green	Light green	Green	Green	Erect	Decumbent
91	barnyardgrass	Monroe	Green	Light green	Green	Light purple	Light green	Green	Horizontal	Open
92	junglerice	Jackson	Brown	Green	Light green	Green	Green	Green	Erect	Open
98	junglerice	Lawrence	Brown	Green	Green	Light purple	Green	Green	Horizontal	Decumbent

Appendix 3.4 Qualitative morphological traits of <i>Echinochloa</i> accessions grown in a common garden at the Main Experiment Station,	
Fayetteville, AR, USA in 2012 and 2013.	

Acc.	Species	County	Caryopsis	Leaf	Node	Inter-	Leaf	Hull	Leaf	Growth
no.			color	sheath color	color	node color	blade	color at maturity	Angle	habit
101	junglerice	Prairie	Brown	Green- purple mixture	Purple	Purple	Green	Green	Horizontal	Decumbent
102	junglerice	Prairie	Green	Green	Light purple	Green- purple mixture	Green	Green- purple mixture	Horizontal	Open
27- Glu	junglerice	Prairie	Brown	Green	Purple	Green- purple mixture	Green	Green	Horizontal	Open
28- Glu	barnyardgrass	Lincoln	Yellow	Green	Light green	Green- purple mixture	Green	Green- purple mixture	Horizontal	Open
34- Glu	barnyardgrass	Lawrence	Brown	Green	Purple	Green- purple mixture	Green	Green- purple mixture	Horizontal	Decumbent
48- Glu	junglerice	Jackson	Brown	Green	Light green	Green- purple mixture	Green	Green	Erect	Open
53- Glu	junglerice	Greene	Green	Green	Purple	Green- purple mixture	Green	Green	Horizontal	Open
71- Glu	junglerice	Phillips	Brown	Green	Purple	Purple	Green	Green- purple mixture	Horizontal	Decumbent
81- Glu	rough barnyardgrass	Jackson	Brown	Green	Green	Green- purple mixture	Green	Green	Horizontal	Decumbent

Acc. no.	Species	County	Upper glume (mm)	Lower glume (mm)	Upper lemma (mm)	Lower lemma (mm)	Spikelet length (mm)	Spikelet width (mm)
1	junglerice	Jefferson	2.68	1.21	2.28	2.63	2.69	1.32
3	barnyardgrass	Ashley	3.35	1.28	3.11	3.33	3.41	1.67
4	coast cockspurgrass	Chicot	2.55	1.21	2.31	2.51	2.58	1.27
5	junglerice	Chicot	2.49	1.23	2.33	2.45	2.50	1.23
6	junglerice	Lincoln	2.60	1.25	2.40	2.54	2.63	1.27
7	junglerice	Monroe	2.45	1.12	2.12	2.40	2.48	1.22
8	junglerice	Arkansas	2.64	1.25	2.45	2.60	2.64	1.28
9	junglerice	Arkansas	2.44	1.12	2.02	2.37	2.46	1.22
10	junglerice	Desha	2.44	1.11	2.22	2.40	2.45	1.20
11	junglerice	Arkansas	2.51	1.23	2.23	2.45	2.53	1.23
12	junglerice	Monroe	2.98	1.42	2.74	2.96	3.00	1.43
13	barnyardgrass	Prairie	2.94	1.31	2.93	2.94	2.95	1.45
14	junglerice	Prairie	2.60	1.28	2.38	2.60	2.62	1.26
15	junglerice	Monroe	2.68	1.26	2.45	2.63	2.70	1.29

Acc.	Species	County	Upper glume	Lower glume	Upper lemma	Lower	Spikelet	Spikelet
no.			(mm)	(mm)	(mm)	lemma (mm)	length (mm)	width (mm)
16	junglerice	Lee	2.49	1.23	2.33	2.45	2.50	1.23
18	junglerice	Lonoke	2.55	1.21	2.47	2.53	2.57	1.26
19	junglerice	Prairie	2.69	1.24	2.57	2.64	2.69	1.37
20	junglerice	Prairie	2.84	1.06	2.36	2.84	2.84	1.34
21	junglerice	Monroe	2.68	1.30	2.45	2.63	2.70	1.31
22	junglerice	Cross	2.44	1.18	2.20	2.40	2.45	1.20
23	junglerice	Crittenden	2.53	1.24	2.33	2.50	2.55	1.20
24	junglerice	Ashley	2.98	1.30	2.75	2.94	3.00	1.40
25	junglerice	Chicot	2.33	1.16	2.19	2.49	2.51	1.21
26	junglerice	Chicot	2.54	1.23	2.25	2.50	2.59	1.26
27	junglerice	Prairie	2.72	1.27	2.52	2.67	2.77	1.31
28	junglerice	Lincoln	2.44	1.18	2.20	2.40	2.45	1.20
29	rough barnyardgrass	Lawrence	4.39	1.29	3.67	4.36	4.40	1.84

Acc.	Species	County	Upper glume	Lower glume	Upper lemma	Lower	Spikelet	Spikelet
no.			(mm)	(mm)	(mm)	lemma (mm)	length (mm)	width (mm)
30	junglerice	Lawrence	2.84	1.06	2.36	2.84	2.84	1.34
31	rough barnyardgrass	Lawrence	3.98	1.61	3.24	3.94	4.01	2.28
32	junglerice	Lawrence	2.50	1.15	2.31	2.47	2.50	1.19
33	junglerice	Lawrence	2.74	1.27	2.57	2.71	2.80	1.29
34	barnyardgrass	Lawrence	3.65	1.28	3.22	3.50	3.79	1.80
35	junglerice	Monroe	2.34	1.12	2.09	2.27	2.38	1.11
36	junglerice	Monroe	2.55	1.21	2.47	2.53	2.57	1.26
37	junglerice	Lonoke	2.55	1.21	2.47	2.53	2.57	1.25
38	junglerice	Cross	2.63	1.23	2.31	2.60	2.65	1.23
39	rough barnyardgrass	Mississippi	4.43	1.65	4.14	4.39	4.43	2.14
40	junglerice	Poinsett	2.66	1.21	2.57	2.64	2.67	1.30
41	junglerice	Mississippi	2.47	1.14	2.26	2.38	2.55	1.22
42	barnyardgrass	Mississippi	3.07	1.27	2.71	3.06	3.10	1.47

Acc.	Species	County	Upper glume	Lower glume	Upper lemma	Lower	Spikelet	Spikelet
no.			(mm)	(mm)	(mm)	lemma (mm)	length (mm)	width (mm)
44	junglerice	Mississippi	2.34	1.1	2.01	2.27	2.38	1.28
45	junglerice	Lawrence	2.55	1.32	2.10	2.48	2.62	1.30
46	rough barnyardgrass	Clay	4.71	1.65	3.60	4.71	4.73	2.27
47	junglerice	Jackson	2.55	1.21	2.47	2.53	2.57	1.26
48	junglerice	Jackson	2.48	1.40	2.33	2.46	2.55	1.20
49	rough barnyardgrass	Greene	4.62	2.51	4.23	4.59	4.62	2.21
50	rough barnyardgrass	Greene	3.50	1.33	3.11	3.31	3.50	1.82
51	junglerice	Greene	2.62	1.17	2.56	2.60	2.64	1.24
52	junglerice	Greene	2.58	1.21	2.33	2.53	2.63	1.30
54	junglerice	Greene	2.80	1.32	2.54	2.74	2.86	1.39
55	junglerice	Prairie	2.33	1.16	2.19	2.49	2.51	1.22
56	junglerice	Prairie	2.44	1.13	1.91	2.35	2.57	1.26
57	junglerice	Prairie	2.63	1.23	2.31	2.60	2.65	1.23

Acc.	Species	County	Upper glume	Lower glume	Upper lemma	Lower	Spikelet	Spikelet
no.			(mm)	(mm)	(mm)	lemma (mm)	length (mm)	width (mm)
58	junglerice	Lincoln	2.70	1.27	2.47	2.67	2.84	1.44
59	junglerice	Prairie	2.43	1.19	2.20	2.40	2.46	1.21
60	junglerice	Prairie	2.57	1.23	2.22	2.54	2.59	1.26
61	junglerice	St. Francis	2.63	1.28	2.31	2.59	2.67	1.30
62	rough barnyardgrass	St. Francis	4.39	2.18	4.11	4.37	4.39	2.43
63	barnyardgrass	White	3.32	1.66	3.18	3.29	3.34	1.49
64	junglerice	White	2.44	1.13	1.91	2.35	2.57	1.26
65	junglerice	White	2.78	1.35	2.40	2.75	2.82	1.31
66	junglerice	White	2.64	1.22	2.37	2.61	2.67	1.30
67	junglerice	Woodruff	2.55	1.21	2.31	2.51	2.58	1.27
68	junglerice	White	2.55	1.21	2.47	2.53	2.57	1.26
69	junglerice	Phillips	2.69	1.25	2.42	2.61	2.71	1.27

Acc. Num.	Species	County	Upper glume (mm)	Lower glume (mm)	Upper lemma (mm)	Lower lemma (mm)	Spikelet length (mm)	Spikelet width (mm)
70	junglerice	Phillips	2.58	1.24	2.26	2.44	2.68	1.33
72	junglerice	Phillips	2.24	1.00	1.98	2.17	2.38	1.28
73	junglerice	Phillips	2.70	1.27	2.47	2.67	2.84	1.44
74	junglerice	Clay	2.56	1.24	2.44	2.53	2.60	1.24
75	junglerice	Phillips	2.43	1.17	2.14	2.39	2.52	1.20
76	junglerice	Drew	2.43	1.11	2.17	2.32	2.58	1.26
77	junglerice	Drew	2.24	0.98	2.13	2.21	2.31	1.12
78	junglerice	Drew	2.23	1.01	2.07	2.15	2.54	1.23
79	junglerice	Poinsett	2.50	1.18	2.32	2.44	2.60	1.27
80	rough barnyardgrass	Poinsett	3.97	1.29	3.22	3.77	4.18	2.01
81	rough barnyardgrass	Jackson	2.95	1.59	2.54	2.94	3.02	1.24
82	barnyardgrass	Jackson	3.34	1.55	3.33	3.32	3.36	1.61
84	rough barnyardgrass	Jackson	3.41	1.39	3.11	3.32	3.41	1.67

Acc.	Species	County	Upper glume	Lower glume	Upper lemma	Lower	Spikelet	Spikelet
no.			(mm)	(mm)	(mm)	lemma (mm)	length (mm)	width (mm)
85	junglerice	Prairie	2.62	1.25	2.44	2.59	2.65	1.28
86	junglerice	Prairie	2.59	1.28	2.38	2.57	2.64	1.29
87	junglerice	Prairie	2.70	1.27	2.47	2.67	2.84	1.44
88	junglerice	Prairie	2.98	1.44	2.42	2.88	3.04	1.43
91	barnyardgrass	Monroe	3.42	1.68	3.20	3.39	3.44	1.62
92	junglerice	Jackson	2.55	1.21	2.47	2.53	2.57	1.26
98	junglerice	Lawrence	2.68	1.3	2.35	2.62	2.72	1.30
101	junglerice	Prarie	2.54	1.17	2.01	2.55	2.61	1.25
102	junglerice	Prairie	2.66	1.14	2.19	2.65	2.72	1.31
27- Glu	junglerice	Prairie	2.77	1.24	2.49	2.72	2.80	1.33
28- Glu	barnyardgrass	Lincoln	3.80	1.57	3.55	3.74	3.80	1.86
34- Glu	barnyardgrass	Lawrence	3.19	1.21	3.04	3.18	3.19	1.59
48- Glu	junglerice	Jackson	2.48	1.4	2.33	2.46	2.55	1.20

Acc.	Species	County	Upper glume	Lower glume	Upper lemma	Lower	Spikelet	Spikelet
no.			(mm)	(mm)	(mm)	lemma (mm)	length (mm)	width (mm)
53-	junglerice	Greene	2.55	1.21	2.47	2.53	2.57	1.26
Glu								
71-	junglerice	Phillips	2.47	1.14	2.26	2.38	2.55	1.22
Glu								
81-	rough	Jackson	3.37	1.65	3.11	3.33	3.44	1.55
Glu	barnyardgrass							

THIS CHAPTER WAS FORMATTED ACCORDING TO

PEST MANAGEMENT SCIENCE

CHAPTER 4

Seed Dormancy Characterization of Echinochloa species

Abstract

BACKGROUND: Dormancy in the *Echinochloa* genus is frequently reported on barnyardgrass (*Echinochloa crus-galli* L.) and junglerice (*Echinochloa colona* L.), but not for other species. Usually, more than one species are present in the same field. A study was conducted in 2013 and 2014 using 94 accessions planted in a common garden in Fayetteville, Arkansas, USA to examine the seed dormancy trait of *Echinochloa* species in Arkansas. Seeds were after-ripened for six months at room temperature (about 25 °C), and then germinated at 32 °C day/ 23 °C night temperature with 12 h light cycle. Germination was recorded at 7 and 14 days of incubation.

RESULTS: Junglerice had the highest germination capacity (41-99%); only 2.7% of the accessions were dormant (2 out of 73 accessions). Barnyardgrass had 56-79% germination capacity; 33% of the accessions were dormant (3 out of 9 accessions). Coast cockspurgrass had 47% germination and rough barnyardgrass had 2-39% germination with all accessions being dormant.

CONCLUSION: Junglerice has the highest fecundity and least dormancy among *Echinochloa* species in the Arkansas-Mississippi River Delta. This trait may have contributed to its dominance among other *Echinochloa* species. Coast cockspurgrass and rough barnyardgrass are expected to persist longer in the soil seedbank compared with junglerice and barnyardgrass because of their high level of dormancy. This trait will impact interspecies population dynamics in response to management tactics.

Nomenclature: barnyardgrass, coast cockspurgrass, junglerice, rough barnyardgrass, Keywords: barnyardgrass, coast cockspurgrass, dormancy-breaking treatment, junglerice, rough barnyardgrass, seedbank

1 INTRODUCTION

Echinochloa is one of the largest genera of the family Poaceae. The genus is comprised of 50 species causing significant yield losses in rice and other crops.¹⁻⁴ In the United States, some *Echinochloa* species were grown as forage for livestock and feed for wildlife.⁵ In Arkansas and the Southern rice belt, *Echinochloa* species are reported as the most important weed in rice production, with barnyardgrass and junglerice also frequently reported among the top ten most common weed in vegetable crops. ⁶⁻⁸ This genus is a major weed in many crops besides rice, including cotton (*Gossypium hirsutum* L.), sugarcane (*Saccharum offcinarum* L.), sorghum (*Sorghum bicolor* L.), peanut (*Arachis hypogea* L.) and cassava (*Manihot esculenta* Crantz).⁹ It is important agronomically and economically to study the dormancy characteristic of these species under the same growing condition because weedy *Echinochloa* can invade and dominate a crop field after just one season and cause significant crop losses.¹⁰

Echinochloa species are prolific seed producers. Barnyardgrass can produce up to 1,000,000 seed per plant depending on environmental conditions. Seed size and weight differ across species and these traits are correlated with seed dormancy.² Studies on dormancy of *Echinochloa* species in the United States have been focused mainly on barnyardgrass and little attention has been given to the dormancy behavior of junglerice (3 studies),¹¹⁻¹³ only one study was conducted on coast cockspurgrass (*Echinochloa walteri* Pursh) and the dormancy of rough barnyardgrass (*Echinochloa muricata* P. Beauv.) has not been studied.¹³ The abundance of

junglerice, rough barnyardgrass, and barnyardgrass, in the Southern United States requires a better understanding of the dormancy behavior of these species. ¹⁴

Echinochloa species exhibit innate (or primary) dormancy at seed maturity, which is terminated when the seed drops from the mother plant. However, some of the seeds will remain dormant for 3-7 months after maturity.¹⁵⁻¹⁶ In general, dormancy can be terminated after prolonged seed storage in dry conditions at room temperature, which is called the after-ripening process.¹⁷ Seeds of many weedy species do not germinate after sufficient after-ripening period; such seeds have some level of secondary dormancy. In the field, many non-dormant seeds are driven into secondary dormancy upon exposure to unfavorable conditions (either high or chilling temperatures, deep burial, too much water, etc.). Seed dormancy in the field can be overcome by modulating soil temperature and light using synthetic or natural ground cover.¹⁸⁻²⁰ Tillage can also encourage germination of weed seeds and allow for control measures of emerged seedlings prior to planting the crop.

Most research on seed dormancy focused on studying environmental conditions or scarification methods to break dormancy. Little is known about interspecies variation in dormancy within the same genus.¹⁷ Further, variation in seed germination behavior among ecotypes of the same species could be high and could impact the efficacy of weed management tactics. For example, the weedy relative of rice (*Oryza sativa* L.) has a wide range of dormancy, with 84-100% germination capacity at 35 °C and a much wider range at lower temperatures.²¹ It is common to have more than one species in a field, which presents a problem in weed management because of variability in germination behavior. ^{17, 22-23}

Barnyardgrass, a specie that looks most similar to junglerice, but with higher dormancy, normally has 5-50% non-dormant seed that can germinate soon after maturation; the rest will

stay dormant and remain in the soil seedbank for a long time.¹⁶ This was confirmed by Kon et al. (2007) who reported that only 39% of fresh deposit of barnyardgrass seeds germinate in the field at one time; the rest can germinate at various times thereafter.²⁴ The resulting extended germination period will be a challenge for farmers in achieving effective season-long weed management.

Light requirement for germination varies across species.¹¹ Light has a strong influence on germination of *Echinochloa* species especially junglerice. Germination of freshly harvested junglerice seed was 43% in the alternation of light/dark and 4% under total darkness; this showed that junglerice is a photoblastic species. A study by Chauhan and Johnson (2009)²⁵ demonstrated that alternating day and night temperatures of 35/25 °C, 30/20 °C and 25/15 °C with 12h photoperiod did not influence the germination of junglerice. Barnyardgrass also requires light for germination. Continuous light resulted in 60% germination of barnyardgrass while only 6% germinated in continuous dark.² A study by Kovach et al. (2010) showed that complete darkness induces secondary dormancy in coast cockspurgrass.¹¹ This means that coast cockspurgrass seed will go into deep dormancy when buried deep in the soil profile where the seed can no longer perceive light. Extreme soil temperature fluctuation is common in temperate countries; this promotes secondary dormancy. Warm temperature in the summer breaks seed dormancy and allows seed emergence.

The efficacy of cultural control practices, like stale seedbed, requires knowledge of variations in emergence patterns, which is dependent upon the dormancy status and germination requirements of seeds. Information on inter-species variation in dormancy of barnyardgrass, rough barnyardgrass, junglerice and coast cockspurgrass will be useful for reducing the soil

seedbank and improving weed control.²⁶⁻²⁷ This study aimed to evaluate the dormancy characteristics of *Echinochloa* species.

2 MATERIALS AND METHODS

2.1 Study Location and Source of Materials

Ninety-four accessions of *Echinochloa* were collected between 2010 and 2011 from rice and soybean fields in 23 counties in Arkansas, USA (Table 4.2). Field experiments were conducted at the Arkansas Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013. The experimental design was a randomized complete block with four replications. Seeds of each accession were planted in nine cells in a 50-cell tray [self-watering capillary tray] filled with Sunshine Mix#1 potting medium [Sun Gro Horticulture, Inc. Bellevue, Washington, USA]. Seedlings were raised in the greenhouse (32/22 °C day/night temperature) from June 20, 2012 and June 14, 2013. The trays were sub-irrigated. Two weeks after planting, seedlings were thinned to two per cell. At the 4-leaf stage (about 3 weeks after planting), seedlings were transplanted manually to the field in a 1.5 m by 1.5 m spacing. Transplanting occurred on July 5, 2012 and June 28, 2013. In 2012, the whole field was sprayed with a mixture of glyphosate (1.12 kg ae/ha) and S-metolachlor (1.79 kg ai /ha) 7 d before transplanting to control other weeds. The field was watered when needed and kept weed-free by hoeing other weeds. In 2013, a mixture of pendimethalin (1.0 kg ai/ha) and S-metolachlor (1.0 kg ai/ha) was sprayed 7 d after transplanting. Nitrogen fertilizer was applied on the day of transplanting at 60 kg N/ha by incorporating the fertilizer around the base of the plant. Insecticide (imidacloprid, 0.22% granule) was applied at 0.20 kg ai/ha to control wireworms. Mature panicles were harvested manually and air-dried for 7 d. Germination experiments were conducted in 2013 and

2014 at the Weed Physiology Laboratory, Department of Crop, Soil, and Environmental Sciences, University of Arkansas-Fayetteville, USA.

2.2 Experiment 1: Pre-germination temperature treatments to break seed dormancy of *Echinochloa colona*

Three accessions, identified as junglerice, were selected randomly out of 94 accessions to represent different seed sizes. The seeds (spikelet consisting of a caryopsis enclosed in lemma, palea, and glume) were threshed manually using sieve #3 [Seedburo Equipment Co. 2293 S. Mt Prospect Road, Des Plaines, IL 60018 USA] for small-seeded accessions. Sieve #4 was used for large-seeded accessions. Threshed seeds were cleaned using a seed blower. Clean seeds were placed in brown paper bags and stored at room temperature (about 25 °C) in the dark for 180 d. Batches of fifty seeds were counted using Elmor C1 seed counter [Firma Elmor, Mangelegg 58, 6430 Schwyz, Schweiz, Switzerland] at a medium speed at the smallest seed size setting, counting 25 seeds/min. The seeds were placed in 9-cm-diameter Petri plates lined with filter paper (Whatman #1). Seeds were incubated for 7 d in four conditions: 1) 50 °C; 2) 4 °C; 3) -20 °C; and 4) ambient temperature (25° C) with four replications per condition. At the end of incubation period, the Petri dishes were arranged, completely randomized, in a tray. Deionized water (10 ml) was added to each Petri plate, and each tray was placed in a clear plastic bag to prevent desiccation. The wrapped trays were then placed in a growth chamber (VWR Diurnal Growth Chamber) at 32 °C day/ 23 °C night temperature with 12 h light provided by four fluorescent tubes. Seed germination was recorded at 7, 14, and 21 d. Seeds were considered germinated when the radicle protruded from the seed coat. At each germination evaluation

period, the germinated seeds were removed from the Petri dish after counting. Seeds with mold were also counted, removed and were excluded from germination calculation.

Germination capacity (%) was calculated using the formula:

Germination capacity = $\frac{\text{total number of seed germinated}}{\text{total number of seed - dead seed (mold-infested)}} \times 100\%$ (Eq. 1)

2.2 Experiment 2: Dormancy evaluation of *Echinochloa* species.

Ten representative panicles were harvested from each field-grown plant (see Section 2.1). The panicles were air-dried in the greenhouse (36 °C) for 7 d, threshed and placed in brown paper bags, and stored at room temperature (25-28 °C) in the dark for 180 d. Ninety-two accessions comprised of 72 junglerice, 9 barnyardgrass, 10 rough barnyardgrass, and 1 coast cockspurgrass were used in this experiment. The seeds were cleaned as described in Section 2.2. The same procedure for seed germination was used as in Experiment 1, except that for this experiment, seeds were stored only at room temperature prior to germination. The germination condition was close to the optimum germination conditions for *E. colona* reported by Chauhan and Johnson (2009) at 30 °C day/ 20 °C night temperature cycle.²⁵ Water was added to the Petri plates every 3 d. Seed germination was recorded at 7 and 14 d of incubation for accessions with high germination capacity. The observation period was prolonged to 21 d for the large-seeded accessions (generally rough barnyardgrass) with low germination capacity. Germination capacity was calculated using Equation 1.

2.3 Data analysis

All statistical analyses were performed using JMP for Windows software (Version 11.0; 2011, Statistical Analysis Systems Institute, SAS Circle, P.O. Box 8000, Cary, NC 25712-8000). To determine the effect of temperature on seed dormancy, an analysis of variance (ANOVA) was conducted on the germination capacity data. The germination capacity of all accessions studied in 2012 and 2013 were pooled in the absence of year effect. Cluster analysis was done on the average germination capacity of each accession to determine statistically supported grouping of accessions based on germination.

2.4 Cluster analysis

The accessions were grouped using the cubic clustering criterion in SAS-JMP (12.1) (figure 4.1). The number of statistically distinct clusters was the point where the cubic clustering criterion reached a maximum, beyond which the curve declines with each additional number of clusters.

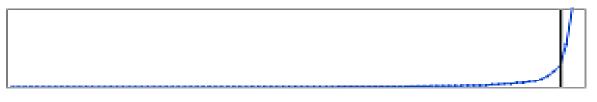


Figure 4.1 Cubic clustering criterion. The point where the cubic clustering criterion reached a maximum, beyond which the curve declines with each additional number of clusters

3 RESULTS AND DISCUSSION

3.1 Dormancy-breaking temperature treatment for junglerice

Temperature is one of the factors that contribute to termination of dormancy.²⁸

Fluctuation of temperature is important in breaking dormancy and inducing germination.²⁹⁻³⁰

Incubation at a constant temperature with low water content in the seed can induce secondary

dormancy.²⁹ This current research focused on junglerice because it is the dominant *Echinochloa* species in the state. In this study, storing the seeds at different temperatures prior to germination, from freezing (-20 °C) to hot (50 °C), did not significantly impact germination capacity (Table 4.1). All pre-germination incubation temperature treatments resulted in 65-77% germination at 32 °C/23 °C day/night temperature with 12 h day length. Incubation at 25 °C (room temperature) for 7 d resulted in 77% germination. Incubation at 4°C for 7 d resulted in 65% germination of the three accessions used in this study. In a germination experiment on junglerice by Chauhan and Johnson (2009) in the Philippines, incubation at room temperature (25 $^{\circ}$ C) also resulted in the highest germination (76%) of junglerice accessions when germinated under 30 °C / 20 °C day/ night temperature with 12 h light. The seeds they used had been after-ripened for 60 d; companion experiments showed that extending the after-ripening period to 90 d did not improve the germination any further. This indicates that in field conditions, ample after-ripening period is a stronger determinant for achieving optimum junglerice emergence during the growing season than temperature. Junglerice can germinate well in a wide range of field temperatures. They also reported that >70% of the seeds germinated when exposed briefly to extremely high temperature i.e., 120 °C for 5 min; germination declined at temperatures higher than this and the seeds were killed at 180 °C.²⁵ This means that, at least for junglerice, seed exposure to high temperatures for short periods in the field would not reduce nor increase the germination capacity compared to 'normal' temperature.

3.2 Species differences in seed dormancy

Among the four species tested, junglerice had the highest average germination capacity (77%) with a range of 41-99% (Table 4.2). The temperature used was within the optimum temperature for junglerice and barnyardgrass germination.²⁵ Barnyardgrass had the second

highest mean germination capacity (53%) with a range of 11-79% across accessions. This was at least twice as high as the germination capacity (25.7±24.9%) reported previously for barnyardgrass var. *crus-galli* and lower than that of var. *oryzicola* (92.9±7.3%) germinated at 30 °C with 12 h photoperiod after 270 d of after-ripening.³² Although direct comparisons with other experiments on various *Echinochloa* species cannot be made, it is informative to mention other reports on germination to obtain an overview of *Echinochloa* germination behavior in a broad range of environments. Brod (1968) reported barnyardgrass germination of 79-86% after 5 mo of storage in room temperature.³³ All previous studies by Kasahara and Kinoshita (1952), Arai and Miyahara (1964) and Brod (1968) showed that 30 °C is the optimum temperature for barnyardgrass seed germination.³³⁻³⁵

In this current study, rough barnyardgrass had the lowest germination capacity (18%) with a range of 2-73% across accessions (Table 4.2). The germination capacity of coast cockspurgrass (49%) was consistent with what was reported by Kovach et al. (2010) under similar germination conditions as the current experiment.³⁶ They also reported that coast cockspurgrass requires both light and dark conditions for germination and does not germinate under complete darkness. Therefore, light is a critical trigger for germination of coast cockspurgrass. This indicates that coast cockspurgrass seed would go into deep dormancy when buried deep in the soil profile where the seed can no longer perceive light. However, the light requirement is species-specific; both junglerice and barnyardgrass can germinate in darkness, but light will increase germination capacity.¹¹ In earlier studies, Buhler and Hoffman (1999) reported that *Echinochloa* species has high dormancy and the germination capacity is variable across species.³⁶

3.3 Inter-species variation in seed dormancy

Echinochloa accessions were separated into three clusters based on germination capacity (Figure 4.1). Cluster 1 was the largest, consisting of 65 accessions (i. 61 junglerice; ii. 3 barnyardgrass; iii. 1 rough barnyardgrass). The average germination capacity of accessions in this cluster ranged from 66-99% (Table 4.3). This was the least dormant group of accessions. The majority (84%) of the junglerice accessions, 33% of barnyardgrass, and 10% of rough barnyardgrass accessions were in this group. The second cluster had 18 accessions (12 junglerice, 4 barnyardgrass, and 2 rough barnyardgrass) with an average germination capacity of 52%. A slightly larger group (44%) of barnyardgrass accessions fell in this intermediate cluster compared to Cluster 1. The third cluster had nine accessions, which was primarily rough barnyardgrass, with only two barnyardgrass accessions. This cluster consisted of the most dormant accessions, with an average germination capacity of 9%. The majority of rough barnyardgrass (70%) were among the highly dormant accessions.

Therefore, junglerice was the least dormant among the three *Echinochloa* species. Given that, about 16% of junglerice accessions showed an intermediate level of dormancy (Table 4.3). The range of germination capacity in Cluster 1 was also notably wide, indicating substantial variation among accessions within the cluster. Such variation may not be statistically significant, but in terms of weed management to reduce the soil seedbank, a 33% difference in germination equates to a large difference in population size and the volume of potential new seed deposit in cases where the population is not controlled effectively. Rough barnyardgrass was the most dormant among the three species and most strongly skewed toward the opposite end of the spectrum in contrast to junglerice. Here, it is tempting to apply the logic of seed size being a factor in seed dormancy.³² However, such hypothesis needs to be supported with genomic data

and is best demonstrated with varying seed size of the same species. The barnyardgrass accessions were spread across the three clusters, with almost equal proportions in Clusters 1 and 2, and a few in Cluster 3. This indicates that barnyardgrass has the greatest diversity in germination behavior among the three species and in that sense, might be the most unpredictable in terms of population response to management tactics.

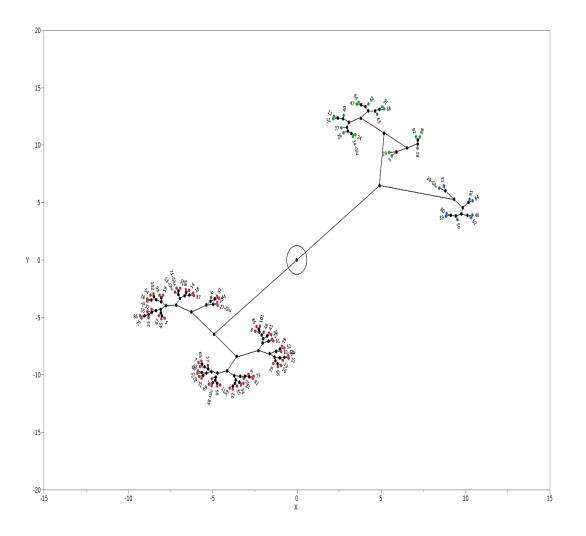


Figure 4.2 Castellation plot showing three clusters of Echinochloa species from Arkansas, USA based on germination capacity. The accessions were grown in a common garden. Seeds were after-ripened for 180 d at 25 °C, and the germination test was conducted in a growth chamber at 32 °C day/ 23 °C night temperature with 12 h light. Cluster 1= red; Cluster 2=green; cluster 3 = blue.

3.4 Intra-species Variation in Seed Dormancy

3.4.1 Cluster analysis of germination capacity of junglerice.

As indicated in the previous section, there was substantial variation in the germination capacity of junglerice although the great majority of accessions were in the low-dormancy category. It turned out that the junglerice accessions, when analyzed without the other species, were separated into four clusters based on germination capacity (Figure 4.2). Cluster 1 (blue) had 46 accessions, with germination capacity ranging from 74-92%, and an average of 83% (Table 4.4). Cluster 2 (red) was comprised of 20 accessions with a germination capacity range of 51-71 % and an average of 63%. Cluster 3 (brown) was comprised of four nondormant accessions with the highest average germination capacity of 97%. This focused analysis showed that the majority (63%) of junglerice accessions had low dormancy, with a few non-dormant accessions. This supports the overall classification of junglerice as the least dormant specie in Section 3.3. Cluster 4 (green) consisted of the two most dormant junglerice accessions were less dormant than the majority of rough barnyardgrass accessions and the most dormant barnyardgrass accessions (see Section 3.3)

3.4.2 Cluster analysis of germination capacity of barnyardgrass.

The nine barnyardgrass accessions separated into two groups (Figure 4.3). Two-thirds of these fell in Cluster 1, with germination capacity ranging from 56-79% with an average of 68% (Table 4.5). One-third of the accessions were more dormant, showing an average germination capacity of 22%, with the highest germination in this group being only 34%. The highest

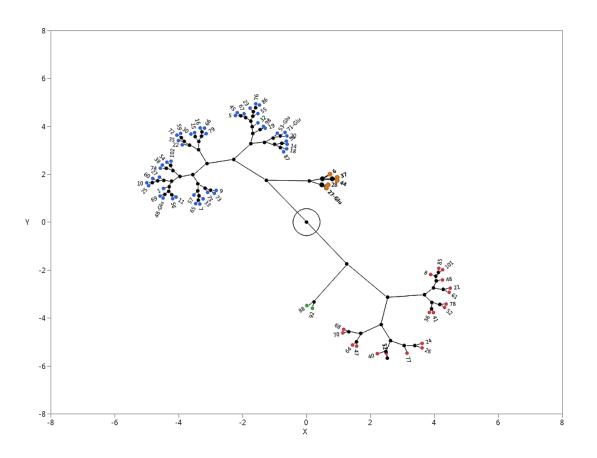


Figure 4.3 Castellation plot showing four clusters of junglerice from Arkansas, USA based on germination capacity. The accessions were grown in a common garden. Seeds were afterripened for 180 d at 25 °C, and the germination test was conducted in a growth chamber at 32 °C day/ 23 °C night temperature with 12 h light. Cluster 1=blue; Cluster 2=red; cluster 3 =brown, Cluster 4 =green.

germination capacity recorded was 79% and the lowest was 11% when germinated under 30/20 $^{\circ}$ C with 8 h of light. The only other report on intra-species variation in germination behavior of barnyardgrass was by Barrett and Wilson (1983). They tested 18 barnyardgrass var. *crus-galli* accessions that were after-ripened for 9 mo and germinated at 30 $^{\circ}$ C with 12 h of light in a two-week period. Eleven accessions had germination capacities between 0 and 25%, four accessions had average germination capacities of 26-50%, two had 51-75% and only 1 had >75% germination.³²

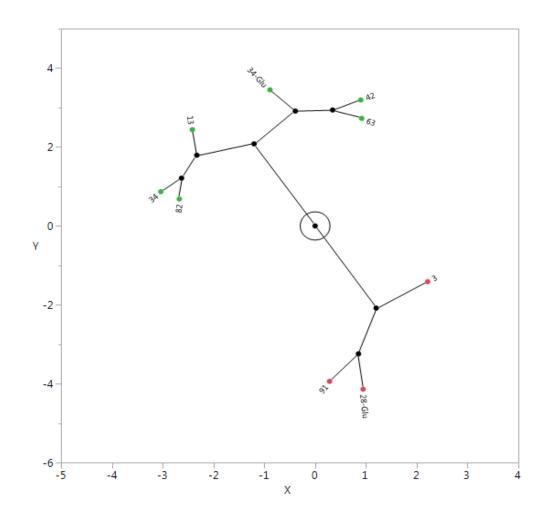


Figure 4.4 Castellation plot showing two clusters of barnyardgrass from Arkansas, USA based on germination capacity. The accessions were grown in a common garden. Seeds were afterripened for 180 d at 25 °C, and the germination test was conducted in a growth chamber at 32 °C day/ 23 °C night temperature with 12 h light. Cluster 1= green; Cluster 2=red.

3.4.3 Cluster analysis of germination capacity of rough barnyardgrass

As discussed previously, rough barnyardgrass had the highest level of dormancy among the *Echinochloa* species tested. The rough barnyardgrass accessions were separated into two clusters based on germination capacity (Figure 4.4). Cluster 1 had seven accessions, with germination capacity ranging from 2-15% and an average of 6% (Table 4.6). The three accessions that composed Cluster 2 had lesser dormancy, showing an average germination capacity of 46%, with the highest germination being 73%. This one accession in Cluster 2 had the highest germination among rough barnyardgrass accessions. The majority of rough barnyardgrass accessions were highly dormant. Considering that this specie also has the largest seeds, it is most likely that rough barnyardgrass will persist for the longest time in the soil compared to junglerice and barnyardgrass. For now, few rice fields in the Arkansas-Mississippi River Delta are infested with rough barnyardgrass. This specie is mostly found in irrigation ditches and field edges. The factors that govern its proliferation in certain niches and its interaction with other *Echinochloa* species in a flooded rice ecosystem are not yet understood.

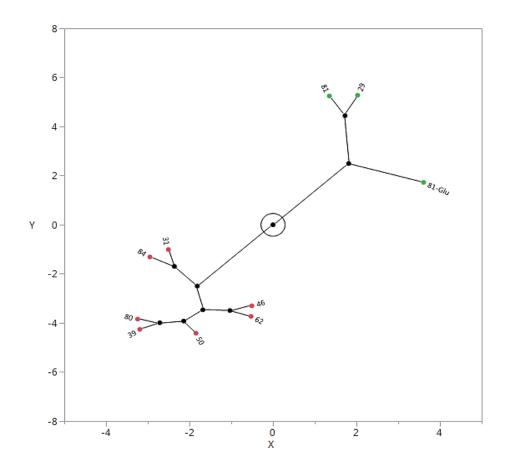


Figure 4.5 Castellation plot showing two clusters of rough barnyardgrass from Arkansas, USA based on germination capacity. The accessions were grown in a common garden. Seeds were after-ripened for 180 d at 25 °C, and the germination test was conducted in a growth chamber at 32 °C day/ 23 °C night temperature with 12 h light. Cluster 1= Red; Cluster 2=green

5

CONCLUSIONS AND RECOMMENDATION

The difference in seed dormancy among and within *Echinochloa* species in Arkansas was significant. Junglerice was the least dormant, and rough barnyardgrass was the most dormant. Seeds with hard seed coat, large seed size, and deep dormancy, such as rough barnyardgrass, are expected to last long in the soil. Variation in germination capacity across species, and among populations within a specie, increases the difficulty of managing *Echinochloa*. Based on this experiment, we can project that junglerice will have the largest batch of seed that could germinate among the first flush of weeds after land preparation. Its persistence, despite its low seed dormancy, is most likely promoted by its high seed production capacity relative to other *Echinochloa* species in the southern USA. Barnyardgrass, rough barnyardgrass, and coast cockspurgrass are expected to have prolonged germination during the crop growing season. We need to educate growers about biological differences among *Echinochloa* species that could impact weed management and resistance evolution. It is important that rough barnyardgrass is controlled whenever it occurs in a field because of the potential longevity of its seed. It would be difficult to eliminate a new invasion.

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Storage	Mean	Std Dev	Min	Max	LSD ^b
condition ^a					
		%			
-20 °C	69	12	47	84	
4 °C	65	13	42	82	9
25 °C	77	10	64	90	9
50 °C	72	10	58	91	

Table 4.1 Germination capacity of junglerice from Arkansas, USA, averaged across accessions, after storage at various temperatures for 7 d.

^a Three junglerice accessions were selected randomly from accessions collected in 2010-2011 and seeds were incubated at -20 °C, 4 °C, 25° C and 50° C for 7 d. Fifty seeds were then germinated, in three replications, at 32 °C/23 °C light/dark cycle with 12 h of light. ^bMeans were compared using Fisher's LSD Test (P=0.05).

Species	Number of Accessions	(Germination capacity ^b				
		Mean	Std Dev	Min	Max		
			%				
junglerice	72	77	13	41	99		
barnyardgrass	9	53	25	11	79		
rough barnyardgrass	10	18	23	2	73		
coast cockspurgrass	1	49					
LSD ^a		11					

Table 4.2 Germination capacity of *Echinochloa* species in Arkansas, USA, (averaged by species).

^aMeans were compared using Fisher's LSD Test (P=0.05).

^bCoast cockspurgrass was not included in the analysis because only one accession was collected. Junglerice, barnyardgrass, rough barnyardgrass and coast cockspurgrass panicles were air-dried in the greenhouse (36 °C) for 7 d, threshed and placed in paper bags, and stored at 25-28 °C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32 °C/23 °C light/dark cycle with 12 h of light.

Accession grouping	No. of	Germination capacity ^b				
	accessions ^a	Mean	Median	Min	Max	
			%			
Cluster 1	65	81	82	66	99	
Cluster 2	18	52	55	27	64	
Cluster 3	9	9	6	2	21	

Table 4.3 Germination capacity of *Echinochloa species* in Arkansas, USA, (averaged across accessions in a cluster).

^a Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.

^bCoast cockspurgrass was not included in the analysis because only one accession was collected. *Echinochloa* species panicles were air-dried in the greenhouse (36 °C) for 7 d, threshed and placed in paper bags, and stored at 25-28 °C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32 °C/23 °C light/dark cycle with 12 h of light.

n Min	n Max
	i Ivian
-%	
74	92
51	71
95	99
41	42

Table 4.4 Germination capacity of junglerice in Arkansas, USA, (averaged across accessions in a cluster).

^a Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.

^bJunglerice panicles were air-dried in the greenhouse (36 °C) for 7 d, threshed and placed in paper bags, and stored at 25-28 °C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32 °C/23 °C light/dark cycle with 12 h of light.

Table 4.5 Germination capacity percentage of barnyardgrass in Arkansas, USA, (averaged across	
accessions in a cluster).	

· a –				
accessions ^a –	Mean	Median	Min	Max
		%		
6	68	69	56	79
3	22	21	11	34
_		6 68	6 68 69	6 68 69 56

^a Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.

^bBarnyardgrass panicles were air-dried in the greenhouse (36 °C) for 7 d, threshed and placed in paper bags, and stored at 25-28 °C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32 °C/23 °C light/dark cycle with 12 h of light.

Accession grouping	No. of		capacity ^b	city ^b		
	accessions ^a -	Mean	Median	Min	Max	
			%%			
Cluster 1	7	6	5	2	15	
Cluster 2	3	46	39	27	73	

Table 4.6 Germination capacity of rough barnyardgrass in Arkansas, USA, (averaged across accessions in a cluster).

^a Four plants per accession were grown in a common garden at the Agricultural Research and Extension Center, Fayetteville, Arkansas, USA in 2012 and 2013.

^bRough barnyardgrass panicles were air-dried in the greenhouse (36 °C) for 7 d, threshed and placed in paper bags, and stored at 25-28 °C in the dark for 180 d. Fifty seeds were then germinated, in three replications, at 32 °C/23 °C light/dark cycle with 12 h of light.

Accession	Treatment	Mean	Std Dev	Min	Max
			% .		
19	4 °C	67	6	62	73
19	-20 °C	76	5	71	82
19	50 °C	77	11	64	91
19	25 °C	76	10	64	87
27-G ^a	4 °C	61	16	44	82
27-G ^a	-20 °C	69	8	61	79
27-G ^a	50 °C	73	11	58	83
27-G ^a	25 °C	86	7	75	90
72	4 °C	65	17	42	82
72	-20 °C	62	16	47	84
72	50 °C	67	8	62	79
72	25 °C	69	6	64	79

Appendix Table 4.1 Germination capacity of selected junglerice accessions in Arkansas, USA, averaged across replications and years stored under various temperature conditions for 7 d prior to germination.

Cluster	Accessions	Species	County	Mean	Std Dev		Max
					·%		
1	1	junglerice	Jefferson	83	4	77	88
1	5	junglerice	Chicot	86	4	82	90
1	6	junglerice	Lincoln	97	3	93	100
1	7	junglerice	Monroe	81	10	70	94
1	8	junglerice	Arkansas	68	11	54	79
1	9	junglerice	Arkansas	79	9	70	87
1	10	junglerice	Desha	82	8	72	90
1	11	junglerice	Arkansas	82	3	80	87
1	14	junglerice	Prairie	89	5	84	97
1	15	junglerice	Monroe	74	8	62	81
1	16	junglerice	Lee	75	5	69	80
1	18	junglerice	Lonoke	89	5	84	96
1	19	junglerice	Prairie	85	10	71	93
1	20	junglerice	Prairie	91	4	87	96
1	21	junglerice	Monroe	66	8	57	75
1	22	junglerice	Cross	78	3	76	82
1	23	junglerice	Crittenden	87	4	82	91
1	25	junglerice	Chicot	82	23	49	100
1	27	junglerice	Prairie	81	5	76	87
1	28	junglerice	Lincoln	95	2	93	98
1	30	junglerice	Lawrence	74	9	63	84
1	32	junglerice	Lawrence	88	9	77	96
1	33	junglerice	Lawrence	80	4	77	86
1	35	junglerice	Monroe	77	13	58	89
1	36	junglerice	Monroe	70	3	67	75
1	37	junglerice	Lonoke	99	2	96	100
1	38	junglerice	Cross	85	3	80	88
1	41	junglerice	Mississippi	70	7	66	80
1	44	junglerice	Mississippi	99	2	97	100
1	45	junglerice	Lawrence	86	4	83	91
1	48	junglerice	Jackson	69	7	59	76
1	52	junglerice	Greene	71	9	60	82
1	54	junglerice	Greene	83	6	77	89
1	55	junglerice	Prairie	87	14	70	98
1	56	junglerice	Prairie	82	5	77	88

Appendix Table 4.2 Germination capacity of *Echinochloa* species in Arkansas, USA, averaged across replications and years.

Appendix Table 4.2 Ger across replications and y	1 /	Echinochloa speci	es in Arkar	nsas, USA	, average	d
Cluster Accessions	Species	County	Mean	Std Dev	Min M	ax

Cluster	Accessions	Species	County	Mean	Std Dev	Min	Max
					%		
1	57	junglerice	Prairie	80	3	77	83
1	58	junglerice	Lincoln	89	6	82	95
1	59	junglerice	Prairie	77	6	69	84
1	60	junglerice	Prairie	81	2	79	84
1	61	junglerice	St. Francis	67	7	60	76
1	65	junglerice	White	81	5	77	88
1	66	junglerice	White	75	10	62	85
1	67	junglerice	Woodruff	87	1	85	88
1	69	junglerice	Phillips	82	10	71	92
1	72	junglerice	Phillips	77	15	56	92
1	73	junglerice	Phillips	79	7	70	86
1	74	junglerice	Clay	84	6	76	89
1	75	junglerice	Phillips	78	7	68	86
1	76	junglerice	Drew	87	1	86	89
1	78	junglerice	Drew	71	10	57	78
1	79	junglerice	Poinsett	75	9	66	88
1	85	junglerice	Prairie	68	7	64	78
1	86	junglerice	Prairie	87	3	83	90
1	87	junglerice	Prairie	88	4	86	94
1	98	junglerice	Lawrence	85	7	79	94
1	101	junglerice	Prairie	68	6	64	77
1	102	junglerice	Prairie	84	3	80	88
1	27-G ^a	junglerice	Prairie	95	6	87	100
1	48-G ^a	junglerice	Jackson	82	3	80	86
1	53-G ^a	junglerice	Greene	90	3	87	93
1	71-G ^a	junglerice	Phillips	92	6	82	96
1	13	barnyardgrass	Prairie	74	3	69	76
1	34	barnyardgrass	Lawrence	78	9	64	84
1	82	barnyardgrass	Jackson	79	3	76	82
1	81-G ^a	rough barnyardgrass	Jackson	73	6	66	80
2	12	junglerice	Monroe	52	1	51	53
2	24	junglerice	Ashley	56	4	52	60
2	26	junglerice	Chicot	57	17	44	80

Cluster	Accessions	Species	County	Mean	Std Dev	Min	Max
					%		
2	40	junglerice	Poinsett	51	16	35	65
2	47	junglerice	Jackson	59	5	53	65
2	51	junglerice	Greene	53	10	47	67
2	64	junglerice	White	60	6	53	68
2	68	junglerice	White	62	3	59	66
2	70	junglerice	Phillips	62	3	60	67
2	77	junglerice	Drew	54	3	50	57
2	88	junglerice	Prairie	41	13	32	50
2	92	junglerice	Jackson	42	3	39	47
2	3	barnyardgrass	Ashley	33	6	25	40
2	34-G ^a	barnyardgrass	Lawrence	56	20	41	86
2	42	barnyardgrass	Mississippi	60	4	57	66
2	63	barnyardgrass	White	64	7	55	71
2	29	rough barnyardgrass	Lawrence	27	6	21	34
2	81	rough barnyardgrass	Jackson	39	5	33	45
2	4	coast cockspurgrass	Chicot	49	14	36	69
3	28-G ^a	barnyardgrass	Lincoln	21	7	14	30
3	91	barnyardgrass	Monroe	11	5	6	18
3	31	rough barnyardgrass	Lawrence	15	5	11	22
3	39	rough barnyardgrass	Mississippi	4	1	2	5
3	46	rough barnyardgrass	Clay	5	4	0	9
3	50	rough barnyardgrass	Greene	2	2	0	4
3	62	rough barnyardgrass	St. Francis	6	3	2	10
3	80	rough barnyardgrass	Poinsett	3	1	2	4
3	84	rough barnyardgrass	Jackson	10	19	0	39

Appendix Table 4.2 Germination capacity of *Echinochloa* species in Arkansas, USA, averaged across replications and years (cont.).

Cluster	Accessions	County	Mean	Std. Dev.		Max	
			%%				
1	1	Jefferson	83	4	77	88	
1	7	Monroe	81	10	70	94	
1	9	Arkansas	79	9	70	87	
1	10	Desha	82	8	72	90	
1	11	Arkansas	82	3	80	87	
1	15	Monroe	74	8	62	81	
1	16	Lee	75	5	69	80	
1	22	Cross	78	3	76	82	
1	25	Chicot	82	23	49	100	
1	27	Prairie	81	5	76	87	
1	30	Lawrence	74	9	63	84	
1	33	Lawrence	80	4	77	86	
1	35	Monroe	77	13	58	89	
1	56	Prairie	82	5	77	88	
1	57	Prairie	80	3	77	83	
1	59	Prairie	77	6	69	84	
1	60	Prairie	81	2	79	84	
1	65	White	81	5	77	88	
1	66	White	75	10	62	85	
1	69	Phillips	82	10	71	92	
1	72	Phillips	77	15	56	92	
1	73	Phillips	79	7	70	86	
1	75	Phillips	78	7	68	86	
1	79	Poinsett	75	9	66	88	
1	48-G ^a	Jackson	82	3	80	86	
2	8	Arkansas	68	11	54	79	
2	21	Monroe	66	8	57	75	
2	36	Monroe	70	3	67	75	
2	41	Mississippi	70	7	66	80	
2	48	Jackson	69	7	59	76	
2	52	Greene	71	9	60	82	
2	61	St. Francis	67	7	60	76	
2	78	Drew	71	10	57	78	
2	85	Prairie	68	7	64	78	

Appendix Table 4.3 Germination capacity of junglerice in Arkansas, USA, averaged across replications and years, by Cluster.

			Mean	Std. Dev.		Max
Cluster	Accessions	County		·%		
2	101	Prairie	68	6	64	77
3	5	Chicot	86	4	82	90
3	6	Lincoln	97	3	93	100
3	14	Prairie	89	5	84	97
3	18	Lonoke	89	5	84	96
3	19	Prairie	85	10	71	93
3	20	Prairie	91	4	87	96
3	23	Crittenden	87	4	82	91
3	28	Lincoln	95	2	93	98
3	32	Lawrence	88	9	77	96
3	37	Lonoke	99	2	96	100
3	38	Cross	85	3	80	88
3	44	Mississippi	99	2	97	100
3	45	Lawrence	86	4	83	91
3	54	Greene	83	6	77	89
3	55	Prairie	87	14	70	98
3	58	Lincoln	89	6	82	95
3	67	Woodruff	87	1	85	88
3	74	Clay	84	6	76	89
3	76	Drew	87	1	86	89
3	86	Prairie	87	3	83	90
3	87	Prairie	88	4	86	94
3	98	Lawrence	85	7	79	94
3	102	Prairie	84	3	80	88
3	27-G ^a	Prairie	95	6	87	100
3	53-G ^a	Greene	90	3	87	93
3	71-G ^a	Phillips	92	6	82	96
4	12	Monroe	52	1	51	53
4	24	Ashley	56	4	52	60
4	26	Chicot	57	17	44	80
4	40	Poinsett	51	16	35	65
4	47	Jackson	59	5	53	65
4	51	Greene	53	10	47	67
4	64	White	60	6	53	68

Appendix Table 4.3 Germination capacity of junglerice in Arkansas, USA, averaged across replications and years (cont.).

Cluster	Accessions	County	Mean	Std. Dev.	Min	Max	
			%%				
4	68	White	62	3	59	66	
4	70	Phillips	62	3	60	67	
4	77	Drew	54	3	50	57	
4	88	Prairie	41	13	32	50	
4	92	Jackson	42	3	39	47	

Appendix Table 4.3 Germination capacity of junglerice in Arkansas, USA, averaged across replications and years (cont.).

Cluster	Accessions	County	Mean	Std Dev	Min	Max	
			%%				
1	13	Prairie	74	3	69	76	
1	34	Lawrence	78	9	64	84	
1	34-G ^a	Lawrence	56	20	41	86	
1	42	Mississippi	60	4	57	66	
1	63	White	64	7	55	71	
1	82	Jackson	79	3	76	82	
2	3	Ashley	33	6	25	40	
2	28-G ^a	Lincoln	21	7	14	30	
2	91	Monroe	11	5	6	18	

Appendix Table 4.4 Germination capacity of barnyardgrass in Arkansas, USA, averaged across replications and years.

Cluster	Accessions	County	Mean	Std. Dev.	Min	Max
				%		
1	29	Lawrence	27	6	21	34
1	81	Jackson	39	5	33	45
1	81-G ^a	Jackson	73	6	66	80
2	31	Lawrence	15	5	11	22
2	39	Mississippi	4	1	2	5
2	46	Clay	5	4	0	9
2	50	Greene	2	2	0	4
2	62	St. Francis	6	3	2	10
2	80	Poinsett	3	1	2	4
2	84	Jackson	10	19	0	39

Appendix Table 4.5 Germination capacity of rough barnyardgrass in Arkansas, USA, averaged across replications and years.

OVERALL SUMMARY

Junglerice (*E. colona*) is the most abundant *Echinochloa* species in Arkansas. Resistance to rice herbicides among *Echinochloa* is widespread in the state, but none has been attributed to junglerice. Due to confusion in species identification, *E. colona*, and *E. crus-galli* are collectively called barnyardgrass and resistance to herbicides has been historically attributed only to barnyardgrass (*E. crus-galli*). The species distribution reported in this study might not reflect the actual distribution of *Echinochloa* species across Arkansas because this study encompassed only rice and soybean fields with remaining infestation at toward crop maturation.

Junglerice is slightly different from barnyardgrass, being shorter in stature, with shorter and relatively erect leaves, and more tillers. Barnyardgrass has intermediate characteristics between rough barnyardgrass and junglerice in terms of overall plant size, tiller numbers and leaf size. The morphological separation among the three species reflects species differentiation in the field because these were grown in a common garden. However, plant height, number of tillers, and leaf length would be lesser *per se* in crop fields or fallow fields where competition from crops or other plants is intense. The diversity in growth habits is also less apparent in crop fields than in open areas. *Echinochloa* in crop fields generally have open to erect canopies because of above ground competition with other plants. Without competition, barnyardgrass and junglerice have ecotypes that exhibit prostrate, decumbent, open, or erect shoot growth. Rough barnyardgrass generally remain erect, but with more open canopy than is observed in crop fields. Junglerice florets are awnless, but barnyardgrass and rough barnyardgrass may or may not have awns. Barnyardgrass and rough barnyardgrass also show variations within a panicle where some spikelets have awns while others are awnless. This variability within a plant makes it difficult to distinguish varieties within a species. It is not possible to differentiate between junglerice and

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barnyardgrass species at the seedling stage, but rough barnyardgrass can be distinguished from other species by its large size. At the vegetative stage, some qualitative traits like leaf angle, growth habit, node color and inter-node color may help in partially identifying the species. Further research should look into tools for species identification at the seedling stage; and whether the differential response to cultural and chemical management exists between species.

Seed characteristics are the most reliable traits to differentiate *Echinochloa* species. Seed length and width; and size of upper glume, lower glume, upper lemma and lower lemma were significantly different among junglerice, barnyardgrass, and rough barnyardgrass. Rough barnyardgrass has a wide range in seed length and width due to the presence of two varieties (var. *muricata* and var. *microstachya*). Overall, there is also a wide range of quantitative traits, resulting in an overlap between species. The wide range of traits indicates the existence of different ecotypes within a specie.

Junglerice is the least dormant among the three species and rough barnyardgrass is the most dormant. The high level of dormancy exhibited by a large group of barnyardgrass, and almost all rough barnyardgrass accessions allow these two species to escape weed management tactics, or survive harsh environmental conditions and persist long in the soil seedbank. Short dormancy showed by junglerice will enable cultural control practices like the stale seedbed approach to be implemented to reduce soil seedbank quickly. The occurrence of multiple species in a field will be a problem because of variation emergence periods, emergence patterns, or dormancy among the species. In this manner, some plants usually are bound to escape weed management tactics and replenish the soil seedbank. Variable dormancy and emergence are also major contributing factors to selection for resistance to chemical weed control practices.

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