DISTRIBUTION, ECOLOGY AND MANAGEMENT OF PROBLEMATIC WEEDS IN RICE PRODUCTION IN TEXAS

A Dissertation

by

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ABSTRACT

Weeds compete with rice for nutrition and other resources, causing yield and economic losses. Herbicides serve as an essential tool for weed management in rice. However, with repeated use of herbicides with the same site of action (SOA), herbicide-resistant weed biotypes have evolved in rice production systems in the U.S. Limited information was available on the herbicide resistance status in Texas rice fields. A paper-based survey was conducted in 2016-17 among the stakeholders to understand their perspective on herbicide use and herbicide-resistance issues. Further, field surveys were carried out to map the distribution of herbicide-resistant *Echinochloa* spp. and weey rice (*Oryza sativa*). Fifty-four *Echinochloa* populations collected from the surveys were planted in a field to understand the evolutionary changes in their morphological and physiological characteristics. Field surveys indicated that junglerice (*E. colona*), weedy rice, and Nealley's sprangletop were the dominant weed species in Texas rice production. In general, barnyardgrass and rough barnyardgrass ecotypes were tallest and exhibited wider flag leaves and longer panicles when compared with the junglerice. Plant height, flag leaf length, seed shattering and seed germination were the highest contributing factors to the diversity of *Echinochloa* ecotypes. The qualitative trait, stem color was highly correlated (0.81) with canopy structure. The *Echinochloa* ecotypes (e.g. *E. colona*) with purple stem color had open geometry with shorter stature compared with other ecotypes (e.g. *E. cruss-galli* and *E. muricata*) with green stem. Surveyed populations (*Echinochloa* spp.) exhibited resistance to imazethapyr, quinclorac, fenoxaprop and propanil, and some populations even exhibited multiple- resisance to more than one herbicide SOAs. However, no significant association was observed between multiple resistance and 13 morpho-physiological traits of characterized *Echinochloa* ppopulations. The findings of this study can help in identifying and characterization of *Echinochloa* spp. in general and devising an alternate herbicide program to control herbicide- resistant weeds.

DEDICATION

This dissertation work is dedicated to my extended family in Ci Shan and Zhang Shan Counties, P.R. China. Thank you for showing me the beautiful scenery in the countryside and planting a seed of agriculture in me.

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CHAPTER I

INTRODUCTION

Rice (*Oryza Sativa* L.) is one of the major crops and a staple food in many parts of the world (Rao et al. 2007). It is the second most widely grown food crop worldwide, only after wheat (FAO 2015). About 90% of the world's rice is produced in Asia (USDA-ERS 2018), which is also the home to half of the world's population. In 2017, the United States (U.S.) produced 7.6 metric tons (MT) of milled rice, which was 1.5% of the total global rice production (USDA-FAS 2018). Nevertheless, the productivity of rice in the U.S. (8.1 MT ha^{-1}) is almost twice as much as the world average (4.5 MT ha^{-1}).

In the U.S., rice is mainly grown in five southern states (Arkansas, Missouri, Mississippi, Louisiana and Texas) and the Sacramento Valley of California (USA rice 2018). The majority of rice produced in the southern region is long grain rice, whereas short- and medium-grain rice are predominantly produced in California (USDA-ERS 2017; Singh et al. 2017). Rice acreage in the U.S. includes both conventional and the imidazolinone herbicide-tolerant rice (Clearfield® rice). The Clearfield® rice technology was developed by BASF Corporation and became commercially available in 2002 (Bollich et al. 2002). In Texas, rice cultivation originally started for domestic consumption, but commercial production began later in the 1880s. Currently, Texas is the fifth largest rice-producing state in the U.S. with an area of about 70,000 hectares planted and 583,000 MT harvested in 2017 (USDA-NASS 2018). About 25% of the total rice acreage in Texas is planted with Clearfield® rice cultivars. There are two major rice producing regions in Texas: areas west of Houston surrounding El Campo, and the areas east of Houston around

Beaumont. Colorado, Wharton, and Matagorda counties are the top three rice-producing counties in Texas, comprising about 60% of the total rice produced in the state (Pack 2017).

Weed management is a major challenge for rice production worldwide. Weeds can compete with rice and cause significant crop yield loss (Oerke 2006). For example, barnyardgrass [*Echinochloa* crus-galli (L.) P. Beauv.] can cause rice yield loss up to 79% at a density of 269 plants m^2 (Smith 1983). In the southern U.S., almost all of the rice is direct-seeded (dry seeded, with delayed flooding at 5 to 6-leaf stage) and the production system is highly mechanized (Hill et al. 1991; Rao et al. 2007). Weeds that are closely related to the biology and morphology of rice are particularly problematic in the direct-seeded rice production systems. The risk of yield loss due to weed competition is higher in direct-seeded, delayed flooded rice compared to the puddled-transplanted rice which is established under flooded conditions (Rao et al. 2007).

The prominent weed species in Texas rice are barnyardgrass, junglerice [*E. colona* (L.) Link], broadleaf signalgrass [*U. platyphylla* (Munro ex C. Wright) R.D. Webster], Nealley's sprangletop (*Leptochloa nealleyi* Vasey), weedy rice (*Oryza sativa*), purple nutsedge (*Cyperus rotundus* L.), yellow nutsedge (*C. esculentus* L. var. *esculentus* L.), and hemp sesbania [*Sesbania herbacea* (Mill.) McVaugh]. Herbicides are the major component of weed management programs in Texas. A typical weed control program in direct-seeded rice systems consists of a burndown herbicide prior to planting, a preemergence (PRE) application immediately after planting, and/or postemergence (POST) herbicides after crop establishment, which include earlypost (EPOST), midpost (MPOST), preflood (PREFLD) and postflood (POSTFLD) applications. However, an injudicious herbicide use has led to rapid evolution of herbicide resistance in several weed species in rice production systems in Texas.

I.1 The biology of dominant weeds in Texas rice production

I.1.1 Echinochloa spp.

Weed species that belong to the genus *Echinochloa* are the most problematic in rice production systems worldwide. The genus *Echinochloa* comprises about 50 weed species that are mostly found in tropical and warm regions (Michael 1983). The *Echinochloa* spp. are highly adaptive and problematic because of their high seed production, seed dormancy, and genetic diversity (Lopez-Martinez et al. 1999; Maun and Barrett 1986). Barnyardgrass is an erect growing weed and can grow up to 2 m tall. It is an annual grass weed, very competitive with rice and can be found in crop fields or along the field edges and roadsides. The leaves of barnyardgrass are linear to lanceolate, with a length of up to 40 cm, and a width of 0.5-1.5 cm (Chin 2001). This weed looks very similar to rice at the seedling stage. However, by the time it differentiates its morphology from rice, crop yield loss may already have occurred (Holm et al. 1977). Junglerice, very similar to barnyardgrass in appearance, is an annual grass but sometimes can behave as a short-lived perennial. It has a prostrate growth habit and can grow up to 0.6 m in height (Hruševar et al. 2015). The seedlings of junglerice are glabrous. The leaves of this weed are lanceolate in shape (Zimdahl et al. 1989), and stems are green or purplish, often branched at the base. Rough barnyardgrass [*E. muricata* (P. Beauv.) Fernald] is another species found in the U.S. rice production. It has diverse phenotypic characteristics compared to other

Echinochloa spp. mentioned earlier. It is often erect and grows about 0.8 to 1.6 m tall. The stem nodes are hairless or have sparse hairs on it (Gould et al. 1972).

The taxonomy of *Echinochloa* genus is highly complex (Lopez-Martinez et al. 1999). The formation of intergrading polymorphic complexes making it difficult to classify this genus (Barrett and Wilson 1981; Sparacino et al. 1994). The species within *Echinochloa* can often be misidentified since this genus lacks conspicuous identification characteristics (Costea and Tardif 2002). The occasional outcrossing is sufficient for gene exchange to occur among the populations (Bagavathiannan and Norsworthy 2014; Maun and Barrett 1986). Five major species of *Echinochloa* have been reported to occur in rice cropping systems worldwide are *E. crus-galli, E. phyllopogon, E. oryzicola, E. oryzoides,* and *E. colona* (Damalas et al. 2008; Gaines et al. 2012; Kaya et al. 2014; Lopez-Martinez et al. 1997; Michael et al. 1983). The genus *Echinochloa* has high inter- and intra-specific diversity, which gives rise to several different ecotypes (Tahir 2016). This makes it difficult to identifying a particular species of *Echinochloa* based on just morphological characteristics.

In Arkansas, in contrast to what has been documented in the literature, junglerice was identified as the most common species, comprising about 80% of the total populations collected from different parts of the state (Tahir 2016). Similarly, in Texas rice, junglerice is thought to be the most dominant of all *Echinochloa* species (Bagavathiannan, personal observations), but a systematic investigation has never been conducted. Such knowledge is valuable for developing suitable management considerations. Therefore, proper identification and reporting of the distribution of *Echinochloa* spp. in Texas is imperative.

I.1.2 Weedy rice (Oryza sativa L.*)*

Weedy rice is another noxious weed in rice production. It is very difficult to get rid of this weed because of its similarity to cultivated rice in morphological, physiological, and biochemical characteristics. The weedy rices commonly found in the southern U.S. have a red pericarp (Gealy et al. 2002; Singh et al. 2000) and thus is called ‘red rice’. Weedy rice was first reported in the U.S. rice production in the mid-1840s (Allston 1846), and later found in all rice growing regions in the U.S. (Burgos et al. 2008; Kanapeckas et al. 2017). It can cause 5 to 80% grain yield loss in rice and has been regarded as one of the most troublesome noxious weeds in the U.S. rice production (Burgos et al. 2008; Nadir et al. 2017). Weedy rice, in general, is taller than rice (0.5 m to 1.7 m height), highly competitive, has high levels of seed shattering and dormancy, and exhibits extended seedbank persistence (Goss and Brown 1939; Noldin 1995).

I.1.3 Nealley’s sprangletop (Leptochloa nealleyi Vasey*)*

Nealley’s sprangletop (*Leptochloa nealleyi* Vasey) is a summer annual grass weed that can grow 1- to 1.5-m tall. This species is predominantly found along the roadsides in southern Louisiana and Southeast Texas but has recently been reported as a problematic weed in rice fields (Bergeron et al. 2015). Nealley’s sprangletop grows erect and has flat stems. Its leaf blades are elongate and flat to loosely spreading (Hitchcock and Chase 1951). It has small hairs on leaf sheath, with a membranous ligule. The seedhead is compact (known as ‘tighthead’) and narrow in shape, with about 25 to 51cm long and about 2.5 to 3.8 cm wide (Webster et al. 2009). The seed of Nealley’s sprangletop is obtuse and very small, with about 1 to 1.5 mm long (Bergeron et al. 2015). Nealley’s sprangletop can sometimes survive through the winter and regrow during the summer, which indicates a short-lived perennial growth habit (Bergeron 2017).

I.2 Important herbicides used in rice production

Clomazone, quinclorac, propanil and fenoxaprop are the most commonly used herbicides in conventional rice production throughout the southern U.S. In Clearfield® rice production systems, imazethapyr is the most commonly used herbicide. The current usage of these herbicides and spectrum of activity on weeds are dependent on the level of resistance to these herbicides.

Clomazone (WSSA Group 13), a PRE-residual herbicide, was introduced to rice production in the 1990s. It is generally used to control annual grasses such as barnyardgrass, broadleaf signalgrass, and sprangletops. It can also suppress some broadleaf weeds including northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] and hemp sesbania. A microencapsulated formulation of clomazone was introduced in 1995 to reduce the volatility and off-target exposure, which enabled clomazone to be applied to the soil surface (Bollich et al. 2000). Clomazone is typically applied alone as PRE or applied in combination with other POST herbicides to provide extended weed control (Zhang et al. 2005).

Quinclorac belongs to the quinoline carboxylic acid family (Group 4). The mechanism of action for this herbicide is not clear, but it acts in a manner similar to the synthetic auxins (Shaner 2014). Quinclorac provides control over annual grass weeds [e.g. barnyardgrasss, large crabgrass (*Digitaria sanguinalis* (L.) Scop.) and junglerice] and broadleaf weeds [e.g. eclipta (*Eclipta prostrata* L.), northern jointvetch and hemp sesbania]. It can also control perennial broadleaf weeds such as field bindweed (*Convolvulus arvensis* L.) and hedge bindweed [*Calystegia sepium* (L.) R. Br.]. Quinclorac can be used both as a PRE and POST option in rice. In susceptible grass plants, it can cause rapid chlorosis at the elongation zone in newly expanding leaves, followed by chlorosis and necrosis of the entire leaves (Shaner 2014).

Propanil, a PS II-inhibiting (Group 7) herbicide, has been one of the most effective grass herbicides in rice production for many years, mainly because of its excellent selectivity between rice and grass weeds. Propanil can control annual grass weeds such as barnyardgrass, broadleaf signalgrass and goosegrass [*Eleusine indica* (L.) Gaertn.]. It can also control annual broadleaf weeds such as hemp sesbania, and curly dock (*Rumex crispus* L.). Rice is naturally tolerant to propanil due to the presence of aryl acylamidase, an endogenous enzyme that can hydrolyze propanil into 3, 4-dichloroaniline, a non-phytotoxic form (Baltazar et al. 1994). The symptoms of propanil injury include leaf chlorosis followed by foliar desiccation and necrosis (Shaner 2014). For almost 30 years, weed control programs in the Southern U.S. were dependent on propanil (Smith and Hill 1990). During that period, about seventy percent of U.S. rice have been applied with propanil at a rate of 3.4 kg ha^{-1} annually. Propanil is still used as an important herbicide in rice production, though its effectiveness has drastically declined due to the evolution of herbicide-resistant weeds.

Fenoxaprop belongs to the aryloxyphenoxy propionate family (Group 1) (Shaner 2014). This family includes a group of herbicides that can inhibit the function of the actyle-CoA carboxylase (ACCase) enzyme, whose function is to catalyze the first step in de novo fatty acid synthesis (Burton et al. 1989). These herbicides can block the production of phospholipids and interrupt the formation of new membranes used for cell growth. Therefore, injured plants often show symptoms such as cessation of tissues at the point of growth and leaf chlorosis. Fenoxaprop is only effective on grass weeds. It was first used in soybean because broadleaf plants are naturally tolerant to this herbicide (Shaner 2014). Rice is also naturally tolerant to fenoxaprop (Stoltenberg 1989), thus it is used in rice production to control grass weeds such as barnyardgrass, broadleaf signalgrass and several sprangletop species.

Imazethapyr belongs to the Imidazolinone family (Group 2). It inhibits the synthesis of the acetolactate synthase (ALS) enzyme, which is responsible for the production of branched-chain amino acids such as isoleucine, leucine and valine (Shaner 2014). Imazethapyr is commonly used in the herbicide-tolerant Clearfield® rice system primarily for controlling weedy rice and volunteer rice. The susceptible plants show reduced growth, leaf chlorosis, and necrosis at 1 to 2 weeks after application (Shaner 2014).

I.3 Herbicide-resistant weeds in rice production

Herbicides serve as an important tool for weed management in rice. However, with the repeated use of herbicides of the same site of action (SOA), herbicide-resistant weed biotypes have evolved in rice production systems (Norsworthy et al. 2013). Currently, rice is among the top three crops with the highest number of herbicide-resistant weed species worldwide (Heap 2018). Among the 51 resistant weed species reported in rice, 41 are resistant to the ALS-inhibiting herbicides, 9 are resistant to the synthetic auxins and the ACCase-inhibiting herbicides, 3 are resistant to the lipid synthesis-inhibiting herbicides, and 1 is resistant to the photosystem II (PS II) - and the long chain fatty acid-inhibiting herbicides. Some weed species are resistant to more than one herbicide SOA. The current knowledge of herbicide resistance in *Echinochloa* spp., weedy rice and Nealley's sprangletop, and the most problematic weeds in the region are provided below.

I.3.1 Echinochloa spp.

The occurrence of herbicide-resistant *Echinochloa* have been reported in major rice producing states in the U.S. (Heap 2018). Barnyardgrass was first reported to evolve resistance to propanil in Arkansas in 1990 (Carey et al. 1995). Resistance to this herbicide was subsequently reported in Texas in 1991, in Missouri in 1994, and in Louisiana in 1995 (Heap 2018). Quinclorac was first introduced in Arkansas rice production in 1992, mainly to control propanil-resistant barnyardgrass when the resistance was prevalent in the southern U.S. rice, but soon it became a widely used replacement for propanil (Malik et al. 2010). In a few years later, barnyardgrass resistance to quinclorac was reported in Louisiana rice in 1998 (Heap 2018). Apart from barnyardgrass, other major species of *Echinochloa*, such as junglerice and late watergrass [*E. Phyllopogon* (Stapf) Koso-Pol.], have also been found resistant to propanil and several other herbicides commonly used in rice (Fischer et al. 2000). In California, late watergrass is the dominant *Echinochloa* species found in rice production. This species was reported to develop resistance to fenoxaprop and thiobencarb, a lipid synthesis-inhibiting herbicide (Group 8) (Heap 2018). Its widespread resistance to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)-, ACCase- and PS II-inhibiting herbicides has already been documented (Heap 2018).

Resistance to multiple herbicide SOA is a growing issue in barnyardgrass in the U.S. rice production, which greatly reduced the number of effective herbicide options available for weed control in rice. The first case of multiple-resistant barnyardgrass was reported in 1999 in Arkansas, with resistance to both propanil and quinclorac (Lovelace et al. 2000). In 2000, a barnyardgrass population was reported resistant to ACCase- and lipid synthesis-inhibitors in California (Fischer 2000). In 2011, barnyardgrass resistance to four herbicide sites of action (ACCase-, ALS-, PSII-, and cellulose-inhibitors) was reported in Mississippi (Heap 2018). Besides, there were several multiple-resistance cases reported in other *Echinochloa* spp.

I.3.2 Weedy rice

Control of weedy rice in conventional rice production was typically achieved by the application of burndown herbicides prior to the planting of the crop. In Clearfield® rice fields, weedy rice could be effectively controlled by imazethapyr (IMI), without any injury on the Clearfield® rice crop, which provides an alternative option for selective control of weedy rice in cultivated rice fields. The Clearfield® rice cultivars were developed through induced mutagenesis (Tan et al. 2005). Soon after its commercialization, widespread resistance to imazethapyr has surfaced in almost all the Clearfield® rice-growing counties in Arkansas (Singh et al. 2017). The potential for gene flow between Clearfield® rice and weedy rice, and the prevalence of ALS-inhibiting herbicide resistant weed species have reduced the utility of the Clearfield® rice technology in recent years (Gealy et al. 2003; Shivrain et al. 2007).

I.3.3 Nealley's sprangletop

Research on the control of Nealley's sprangletop in rice is very limited due to the fact that it is a fairly new species in the southern U.S. rice production systems. According to a recent study on the evaluation of different herbicides for Nealley's sprangletop control, fenoxaprop is by far the best option, while imazethapyr can only provide some level of suppression (Bergeron et al. 2015). Propanil at 4,480 g ai/ha rate applied alone can control Amazon sprangletop [*Leptochloa panicoides* (J. Presl)] at 87% (Smith 1975) and at the same rate can control bearded sprangletop [*Leptochloa fusca* (L.) Kunth var. *fascicularis* (Lam.) N. Snow] at 62% (Smith and Khodayari 1985), but it is found not effective (38%) on Nealley's sprangletop (Bergeron et al. 2015).

Knowledge of the distribution of herbicide-resistant weeds is imperative for developing effective weed management programs. Currently, little is known about the herbicide resistance status of Texas rice fields. Further, it is important to understand the evolutionary changes in their morphological and physiological characteristics, especially in *Echinochloa* species, given their broad variation in traits.

The objectives of this project were to:

1. Conduct field and stakeholder surveys for problem weed issues in Texas rice;
2. Confirm and characterize herbicide resistance in *Echinochloa* spp., Nealley's sprangletop, and weedy rice;
3. Characterize different *Echinochloa* spp. biotypes collected from Texas rice production systems using morphological features.

The hypotheses underpinning the objectives of this project were:

1. Weed management surveys will reveal the perspective of stakeholders on problematic weeds and management practices in Texas rice production, and will help identify research priorities (Objective 1)
2. Herbicide resistance is prevalent in *Echinochloa* spp., and weedy rice biotypes in Texas rice production (Objective 2)

3. Different *Echinochloa* spp. are present in the rice production regions in Texas and there is high phenotypic diversity among the populations (Objective 3)

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CHAPTER II

STAKEHOLDER AND FIELD SURVEYS ON WEED MANAGEMENT ISSUES AND RESEARCH NEEDS IN RICE PRODUCTION IN TEXAS

II.1 Introduction

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population (Rao et al. 2007). United States (U.S.) produced 7.6 metric tons (MT) of milled rice in 2017, which was 1.5% of the global rice production (USDA-FAS 2018). Rice in the U.S. is mainly grown in five southern states (Arkansas, Missouri, Mississippi, Louisiana, and Texas) and the Sacramento Valley of California (USA rice 2018). The production system is highly mechanized and the vast majority of rice in the southern U.S. are direct-seeded (seeded dry, with delayed flooding at about 5 to 6-lf seedling stage) (Hill et al. 1991; Rao et al. 2007). Currently, Texas is the fifth largest rice-producing state in the U.S., with an area of about 70,000 hectares planted and 583,000 MT harvested in 2017 (USDA-NASS 2018). In Texas, rice is produced in two major regions, the areas in west and east of Houston (Fig. 1).

Weed management is a major production challenge in rice. Problematic weeds commonly known to occur in rice production in Texas include junglerice (*Echinochloa* colona), weedy rice (*Oryza sativa*), sprangletops (*Leptochloa* spp.) and sedges (*Cyperus* spp.). Junglerice is highly adapted to rice growing conditions and is a major weed in rice production worldwide (Holm et al. 1977). Weedy rice is another significant concern and as it is difficult to control in rice because of the morphological, physiological and genetic similarities between the two species. Herbicide-resistant Clearfield® cultivars allow for selective control of weedy rice but potential gene flow from Clearfield rice to weedy rice has limited the use of Clearfield rice technology in the recent years. (Shivrain et al. 2007). Apart from these, a mix of sprangletop species as well as sedges are commonly observed in rice production in Texas. However, no systematic investigations have been carried out so far in rice production in Texas to document the nature and extent of current weed management issues and needs.

A statewide survey of stakeholders can be useful to gather information about current weed management practices, monitoring changes to weed control practices, identifying problematic weeds, and determining research as well as outreach needs (Webster and Coble 1997). For example, Shaw et al. (2009) conducted a grower survey in four midwestern states and two southern states in the U.S., which was useful in collecting information on crop rotation practices, weed control, as well as concerns for herbicide resistance. Likewise, routine weed management surveys conducted in rice, cotton, and soybean production systems in the Midsouthern states have been found to be invaluable for researchers and extension personnel (e.g. Norsworthy et al. 2013; Riar et al. 2013; Schwartz-Lazaro et al. 2018). These surveys are typically carried out using paper-based questionnaires distributed to stakeholders through surface mails and/or during field days and other events. Online surveys are also considered wherever feasible (Regnier et al. 2016). These stakeholders include, but not limited to, growers, crop consultants, industry representatives, county extension agents, university extension scientists, and agrochemical dealers and distributors. Some surveys typically target a specific group of stakeholders, e.g. crop consultants (Riar et al. 2013).

To assess the importance of weed species (both common and problematic) infesting specific production systems, the stakeholder surveys can be combined with actual field surveys to obtain more robust information. While field surveys can reveal common weed escapes, stakeholder surveys can indicate problematic weeds that are difficult-to-control. Field surveys are often carried out during the late-season prior to crop harvest to document weed escapes, which represents weed control issues (Johnson et al. 2004; Leeson et al. 2005). Late-season escapes that occur prior to crop harvest are comprised of weeds that survive control measures during early season and the ones that recruit and establish after all control measures have been terminated. Late-season weed escapes contribute to seedbank persistence, making weed management difficult in the years to come (Bagavathiannan and Norsworthy 2012). Late-season surveys for weed escapes have been invaluable in understanding weed shifts and problematic weeds. A survey conducted in Indiana soybean production showed that late-season escapes of giant ragweed (*Ambrosia trifida*), giant foxtail (*Setaria faberi*), horseweed (*Conyza canadensis*), and other weed species were present in about 97% of the surveyed fields (Johnson et al. 2004). Likewise, field surveys conducted across the Prairie provinces in western Canada revealed the widespread occurrence of late-season weed escapes in many fields (Leeson et al. 2005). Thus, late-season weed surveys will be vital for understanding problematic weed issues in rice production in Texas.

The objectives of this research were to 1) identify common and problematic weeds infesting rice fields, 2) understanding current weed management practices, and 3) prioritize research and educational needs for profitable rice production in Texas.

II.2 Materials and Methods

II.2.1 Stakeholder survey

A one-page survey questionnaire was designed (Table 1) to collect weed management-related information from a broad range of stakeholders involved in rice production in Texas. They included rice growers, consultants, dealers and distributors, sales representatives, and other interested clientle. Survey questionnaires (IRB: IRB2017-0195) were distributed among the stakeholders at the Western Rice Belt production conference (January 2017) and field days at Eagle Lake (June 2015, 2016) and Beaumont (July 2015, 2016). Completed surveys were colleced at the end of the events or through mails.

The questionnaire was made up of 15 questions related to several aspects of crop production and weed management in rice production in Texas. It began with asking the background information such as the role of the respondent, the location and size of the rice farms they oversee, as well as the crop rotation used. The respondents were asked to rank the PRE and POST herbicide programs most often used or recommended by them from a list of 7 PRE options [clomazone (Command®), quinclorac (Facet®), imazethapyr (Newpath®), thiobencarb (Bolero®), pendimethalin (Prowl®), saflufenacil (Sharpen®), or other] and 6 POST options [imazethapyr (Newpath®), quinclorac (Facet®), cyhalofop (Clincher®), propanil (Riceshot®), bispyribac-sodium (Regiment®), fenoxaprop (RiceStar®), or other]. Any herbicide option that was not provided in the list but used/recommended by the respondent was indicated in the ‘other’ option. Points were given for each option (7 = the most often used/recommended PRE-herbicide, and 6 = the most often used/ recommended POST- herbicide; and 1 = least used/ recommended herbicide). An accumulation point was then calculated at the end.

For questions related to problematic weed species, stakeholders were asked to list each species from the most problematic to the least problematic. Points were given on a scale of 5 to 1 where 5 = most problematic, and 1 = least problematic. Total points were calculated for each species for all respondents and then ranked. Information on the acreage of Clearfield® rice (resistant to imidazolinone herbicides) a respondent supervises and the use of herbicides other than those that inhibit the acetolactate synthase (ALS) enzyme in the Clearfield® rice system was collected. Additionally, the number of times in a year field scouting was carried out, and the level of weed infestation in the field (4 levels: very serious, serious, moderate, and none), were obtained. Respondents were asked to select the factors influencing weed control decision including economic threshold, previous experiences, general field appearance, recommendations by the university, and dealer/distributor recommendations. For this question, respondents could choose more than one factor.

Questions were asked about non-chemical weed management practices implemented and challenges encountered. Information on the cost of weed management in rice for both the main and ratoon crops were collected. Further, questions were asked on the level of concerns that the respondents have for herbicide-resistant weeds and suspected herbicide-resistant weed species (including associated herbicides) occurring in their fields. Finally, the respondents were asked to select research topics that they think were important to them. These included improved strategies to control herbicide-resistant weeds, developing new herbicide-resistant rice varieties, economical weed management practices, improving the efficacy of current herbicides, reducing rice injury from herbicides, and preventing soil seedbank. The respondents also had the option of indicating research topics that were not listed in the questionnaire and were encouraged to provide any additional suggestions that would help direct future research and extension efforts.

II.2.2 Field survey

Late-season field surveys were conducted during July-August in 2015 and 2016 across the entire rice growing region in Texas. The survey locations were pre-determined by observing the presence of levees on Google® map across the historical rice growing regions in Texas, using the ITN Converter (Benichou software). The survey sites were randomly selected in the software without prior knowledge of the fields, following a semi-stratified survey methodology (Bagavathiannan and Norsworthy 2016). The way points were converted into an ITN file and loaded into a Global Positioning System (GPS) device (TomTom International) for easy navigation to the pre-determined survey sites. If a rice field was not present or no weed escapes were observed at the pre-determined site, then the first rice field with weed escapes along the route to the next pre-determined site was surveyed. In each survey field, the infestation (%) of each prominent weed species was documented and seed samples were harvested from mature inflorescences for herbicide resistance evaluations. The GPS coordinates of each survey field were also documented.

II.2.3 Data analysis

Answers obtained for the survey questionnaire were analyzed based on frequency distribution. Means and standard error of the means for frequency distribution were calculated using JMP. Ranking was assigned to the treatment means based on the total points of each response received. Spatial maps were developed using ArcGIS (version

10.5; ESRI) to illustrate spatial distribution of prominent weed species across rice production fields in Texas. The distribution of rice weeds and their infestation levels in each field were illustrated using the interpolation analysis technique based on Inverse Distance Weight (IDW). The IDW interpolation determines cell values using a linearly weighted combination of a set of sample points. The occurrence of weedy rice in rice fields was shown using kernel density analysis. The percent occurrence and average density of each weed species were calculated using Equations 1 and 2 (Rankins et al. 2005).

$$\%\ \text{occurence} = \frac{\text{Number of fields infestated}}{\text{Total fields sampled}} * 100 \ \ [1]$$

$$\text{Average density} = \frac{\sum \text{Density from each field where species was present}}{\text{Number of fields where species was detected}} * 100 \ \ [2]$$

II.3 Results and Discussion

II.3.1 Stakeholder survey responses

One hundred and eight out of the 300 survey questionnaires distributed were returned, resulting in a 36% response rate. Rice growers (71% of the respondents) and consultants (6%) comprised most of the respondents, representing an average of 496 and 1,218 hectares of rice production operations, respectively. Colorado and Wharton counties had 26% and 25% of the total respondents, respectively. Colorado, Wharton, and Matagorda are the top three rice-producing counties in Texas, comprising about 60% of the total rice produced in the state (Pack 2017).

II.3.2 Crop rotation

Rice-fallow-rice was the most common rotation practice (55%), followed by rice-fallow-fallow-rice (20%), rice-soybean-rice (12%), and continuous rice (9%). Other rotation practices account for the rest 4% of the fields, including rice-rice-fallow, rice-corn, rice-grain sorghum, and rice-crawfish-rice. Crop rotation is considered an important weed management practice in rice-based systems (Malik 2010). Unlike the Midsouth where soybean [*Glycine max* (L.) Merr.] is the most common rotation with rice (Norsworthy et al. 2013), fallowing is commonplace in Texas. Poor soil drainage and a lack of economically attractive crop option are the drivers for fallowing after rice in such lands. The fallowed lands are typically used for animal grazing, often for two consecutive years and then return to rice in the third year. Research shows that it takes about two years to establish a satisfactory pasture following rice (Bray 1939). Animal grazing can be an effective non-chemical tool for weed management in the rotational years as grazing negatively impacts the persistence of problematic weeds, including herbicide-resistant biotypes. Moreover, the use of herbicides is completely eliminated in the fallow years, thus there is a general reduction in selection pressure for herbicide resistance evolution. Soybean is often rotated with rice in lands with sufficient drainage. About 9% of the fields were continuously grown rice every year, which means the same land was used to grow rice repeatedly.

II.3.3 Weed issues

Stakeholder survey response. Stakeholders were asked to rank the top five most problematic weeds that they dealt with. Considering difficulty in distinguishing the subspecies by stakeholders, some answers were grouped together and presented as one species. For example, both junglerice and barnyardgrass were referred to as "barnyardgrass" (*Echinochloa* spp.). Others included sprangletops (*Leptochloa* spp.), sedges (*Cyperaceae* spp.), pigweeds (*Amaranthus* spp.), dayflower (*Commelina* spp.), and crabgrass (*Digitaria* spp.).

Barnyardgrass (24% of the respondents) and sprangletops (16%) were ranked as the top two most problematic rice weeds by the stakeholders. Both species appeared frequently in the top five most problematic weed species identified by each respondent and ranked the top two based on the weighted score (Table 3). The commonly occurring sprangletops in rice production in Texas included Nealley's sprangletop (*L. nealleyi* Vasey.) and Amazon sprangletop (*L. panicoides*). Sedges were ranked as the third most problematic species by the stakeholders. Some common sedges included yellow nutsedge (*C. esculentus*), purple nutsedge (*C. rotundus*), rice flatsedge (*C. iria*), and smallflower umbrella sedge (*C. difformis*). Weedy rice (*Oryza sativa*) and broadleaf signalgrass (*Brachiaria platyphylla*) were ranked as the 5th and 6th most problematic weed species, respectively.

Alligatorweed and pigweeds were the most problematic broadleaf weeds, ranking 4th and 7th, respectively, among all weed species listed by the stakeholders (Table 3). Alligatorweed has been reported as one of the most troublesome rice weeds in Louisiana and Texas in the early 2000s (Webster 2001). It is an invasive species found in many aquatic environments. It is a perennial species and can grow very fast, with the ability to double biomass in 50 days (Brown and Spencer 1973). The predominantly occurring pigweed species in the rice production areas of Texas was common waterhemp (*A. tuberculatus*). It is often found on the levees or edges of the rice field. Hemp sesbania [Sesbania herbacea (Mill.) McVaugh] and dayflower were ranked 8th and 9th, respectively. Benghal dayflower (*C. benghalensis*) and spreading dayflower (*C. diffusa*) are common in this region.

In total, 17 weed species were mentioned by the stakeholders among the top five most problematic weed species. The list of top ten weeds included four grass weeds, five broadleaf weeds and a sedge. Twenty-two percent of the respondents reported very serious weed infestation in their fields. Thirty-three percent rated the level of weed infestation as "serious", and the rest rated it as "moderate". For the question about the frequency of field scouting, the responses ranged from daily to 3 or 4 times per a cropping season. However, scouting on a "weekly basis" was the most common answer.

Field survey. The occurrence of weed escapes were documented during a field survey conducted prior to rice harvest. These weeds do not necessarily represent problematic weeds but had escaped control measures. Weeds that are typically common may not necessarily be viewed as problematic by the stakeholders if control is not difficult. Conversely, weeds that are not widespread but difficult to control are usually considered problematic by the stakeholders.

The level of late-season weed infestation prior to rice harvest across the Texas rice production belt is shown in Fig. 2a. Junglerice [*E. colona* (L.) Link], Nealley's sprangletop and hemp sesbania were the top three most escaped weeds, with frequency of occurrence of 65, 43 and 31%, respectively (Table 2). Farmers sometimes refer junglerice as "redtop". It had the highest average density (13% field infestation) among all the weed species (Table 2). Field survey showed that junglerice was more prominent than barnyardgrass in rice production fields in Texas. In some fields, junglerice infested about 25% of the entire field area (Fig.2b). Eighteen percent of the surveyed fields had barnyardgrass infestation, with an average density of 5% (Table 2).

Nealley's sprangletop is a fairly new species to rice production in Texas and Louisiana. It is typically found on the roadsides but has moved into rice fields in recent years (Bergeron et al. 2016). Though Nealley's sprangletop was documented very frequently in rice fields, the average densities were low (3%) in this study (Table 2). For hemp sesbania, its average field densities were the second highest, at about 11% (Table 2). High infestations of hemp sesbania in the current field survey could be attributed to organic rice fields where control options were very limited. It is a broadleaf, leguminous weed with a woody stem, which can grow up to 3m tall at maturity (Lorenzi and Jeffery 1987). High competitiveness and shading are the reasons that hemp sesbania causes significant crop yield losses (King and Purcell 1997).

Weedy rice was found in about 10% of the surveyed fields (Fig.3). Though the frequency of occurrence was low, the densities were often high at about 11% average field infestation. The weedy rice ecotypes noted during the survey were usually tall, growing above the canopy of rice. It was observed that the maturity of weedy rice is not coincided with cultivated rice in some areas. Other dominant weed species documented during the late-season field survey included common waterhemp (*Amaranthus tuberculatus* L.), Texasweed [*Caperonia palustris (L.) St. Hil*], northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.], and sedges (Table 2). In general, the late-season weed escapes were greater in the areas west of Houston, particularly in Wharton and Colorado counties, compared to the areas east of Houston.

Weed escapes typically result from inadequate weed control with management operations conducted during the cropping season. For herbicides, factors such as poor spray coverage, inadequate rate, delayed application timing, lack of an adjuvant, wrong combination of tankmix herbicides, and unsuitable environmental conditions, among others, can cause a reduction in efficacy and lead to weed escapes (Hartzler and Battles

2001; Jordan et al. 1997). Weed escapes or poor weed control can also be attributed to herbicide resistance in those populations.

II.3.4 Weed management options

The frequency of use of the listed PRE and POST herbicides was calculated based on total scored points for each herbicide (Fig. 4). Eighty-six percent of the survey respondents (93 of the 108) recommended a pre-emergence herbicide immediately following rice planting. Clomazone [Weed Science Society Association of America (WSSA)-Group 13], was the most frequently used pre-emergence herbicide, with 37% of importance (Fig. 4a). Clomazone was also the most often recommended PRE-herbicide in rice production in Arkansas and Mississippi (Norsworthy et al. 2007, 2013). Clomazone was introduced to US rice production in the 1990s to control annual grasses such as barnyardgrass, broadleaf signalgrass, and sprangletops. It can also suppress some broadleaf weeds including northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.] and hemp sesbania. The microencapsulated formulation of clomazone was developed and introduced in 1995, which enabled its use on the soil surface due to low volatility and off-target movement (Bollich et al. 2000). Clomazone is usually applied alone as PRE, but it can also be tankmixed with other POST herbicides to provide extended weed control (Zhang et al. 2005).

Quinclorac (WSSA Group 4) was the second most popular PRE herbicide, with 19% of importance (Fig. 4a). These findings are consistent with reports in Arkansas rice production, where quinclorac was recommended as the second most often used PRE-herbicide by 40% of the consultants (Norsworthy et al. 2007). The mechanism of action of quinclorac is not clear, but it acts in a manner similar to the synthetic auxins (Shaner

2014). Quinclorac provides control of annual grasses [e.g. barnyardgrass, large crabgrass (*Digitaria sanguinalis* (L.) Scop.), and junglerice] and certain broadleaf weeds [e.g. eclipta (*Eclipta prostrata* L.), northern jointvetch and hemp sesbania]. It can also control perennial broadleaf weeds such as field bindweed (*Convolvulus arvensis* L.) and hedge bindweed [*Calystegia sepium* (L.) R. Br.].

Saflufenacil (WSSA Group 14) was the third most popular herbicide, with 15% of importance (Fig.4a). Saflufenacil inhibits the protoporphyrinogen-IX-oxidase (PPO) enzyme to catalyze the process of producing chlorophyll (Geier et al. 2009; Grossmann et al. 2010). It is used in rice production for controlling broadleaf weeds such as hemp sesbania. Saflufenacil is often tankmixed with other herbicides (e.g. clomazone, imazethapyr) to improve weed control spectrum (Camargo et al. 2011). Pendimethalin (WSSA Group 3) was recommended by respondents with 7% of importance, ranked as the 5th most popular PRE-herbicide. Pendimethalin inhibits seedling root growth by inhibiting microtubule assembly during mitosis. This herbicide is often used as a delayed PRE-option in rice, about 3 to 4 days after rice seeding for controlling grasses and some broadleaf weeds. Results of this survey have indicated that PRE-herbicides are widely used in rice production in Texas, a trend that is consistent with Arkansas and Mississippi rice (Norsworthy et al. 2013). PRE-herbicides serve as the foundation for herbicide resistance management and their continued use is critical (Norsworthy et al. 2007; Norsworthy et al. 2012).

With respect to POST herbicides, quinclorac (22%), propanil (19%), imazethapyr (17%) and cyhalofop (17%) were the popular choices by the respondents (Fig.4b). Quinclorac was preferred because it also provides residual weed control. In Arkansas, quinclorac was recommended by 47% of the rice consultants as a POST herbicide option (Norsworthy et al. 2007). Propanil, a PSII-inhibiting (WSSA Group 7) herbicide, has been used in rice production for many years since its first introduction in 1959 (Smith and Hill 1990). It has an excellent selectivity between rice and grass weeds (Frear and Still 1968). Rice is naturally tolerant to propanil due to the presence of aryl acylamidase, an endogenous enzyme that can hydrolyze propanil into 3, 4-dichloroaniline, a non-phytotoxic form (Baltazar and Smith 1994). Propanil is still used as an important herbicide in rice production, though its effectiveness has drastically declined due to the evolution of resistance in weeds such as barnyardgrass (Baltazar and Smith 1994; Lovelace et al. 2000). Imazethapyr (WSSA Group 2) is an Acetolactate Synthase (ALS) inhibitor herbicide, which inhibits the biosynthesis of branched-chain amino acids isoleucine, leucine and valine by inhibiting the function of the key enzyme acetohydroxyacid synthase (AHAS). It is used in the Clearfield^{TM} rice system for controlling weedy rice and other grass weed species. The current use of POST herbicides in the region is significantly greater compared to their use levels 10 years ago (Norsworthy et al. 2013). The increase in POST herbicide use is consistent with the widespread evolution of resistance in weeds such as barnyardgrass to propanil and quinclorac (Malik et al. 2010).

Implementation of some forms of non-chemical weed control is also common in rice production in Texas. Forty-seven respondents (44%) have indicated that they adopt non-chemical weed control methods such as flooding (36%), tillage prior to planting (49%), stale seedbed (4%), and crop rotation (4%). Three respondents (6%) of the 47 didn’t specify which kind of non-chemical weed control method they used. The stakeholders were also asked to specify the constraints of using non-chemical weed management. Seventy-seven percentage (37 out of 48) of the respondents felt limited non-chemical options is a barrier. Respondents also noted that the non-chemical options were often ineffective (63%), time consuming (58%) and/or expensive to implement (48%).

II.3.5 Factors influencing weed control decision making

Seventy-two of the respondents (57 out of 79) made a weed control decision based on economic threshold (ET), whereas 63% of them based on weed problems from previous years. General appearance of the field was considered by 43% of the respondents for decision making. Forty-eight percent of the respondents made a weed management decision based on recommendations from dealers and 39% of them made decisions based on University recommendations. Approximately 10% of the respondents relied on consultants, agronomists or weed management guides for weed control recommendations. Findings of this survey showed that ET is the top consideration that guides weed management decision making. When decisions are made based on economic threshhold, the late- season escapes may be neglected because they don’t cause direct yield loss in the current year (Bauer and Mortensen 1992). However, the late-season escapes can contribute to soil seedbank and increase management expenses in the years to come

II.3.6 Herbicide-resistant weeds

With respect to the level of concern for herbicide-resistant weeds, 88% (77 out of 87) of the respondents expressed moderate to high concern, and the rest indicated that they had a low level of concern or no concern at all about the evolution of herbicide-resistant weeds in their fields. The high level of concern expressed by the stakeholders suggests that they are already dealing with herbicide-resistant weeds in their fields or observe resistant weeds in their planting areas. Suspected herbicide-resistant weeds listed by the respondents include imazethapyr-resistant weedy rice; propanil-, quinclorac-, clomazone-, and/or imazethapyr- resistant barnyardgrass; glyphosate- resistant sprangletops; and glyphosate- and/or ALS-inhibitor resistant waterhemp.

Herbicide-resistant weeds have been prevalent in rice production in the Midsouthern states for many years. Herbicide-resistant biotypes of weedy rice and barnyardgrass were perceived to be very common in the region (Norsworthy et al. 2013). ALS-inhibitor resistance in weedy rice was documented within few years after the commercialization of Clearfield® rice in Arkansas and has been widespread since then (Singh et al. 2017). The utility of the Clearfield® rice technology has been reduced because of gene flow and transfer of herbicide resistance from Clearfield® rice to weedy rice, as well as the evolution of ALS-inhibitor resistance in other weed species (Gealy et al. 2003; Shivrain et al. 2007). Barnyardgrass was first reported in Arkansas to have evolved resistance to propanil in 1990 (Carey et al. 1995). It was then reported to be resistant to quinclorc in Louisiana rice production in 1998 (Heap 2018). In 2007, clomazone-resistant barnyardgrass was detected in Arkansas (Norsworthy 2007). Subsequently, ALS-inhibitor resistance has also become widespread in this species (Rouse et al. 2018). Herbicide resistance in sprangletops and waterhemp were also raised as a concern, but characterization of field collected samples would provide more insight into the nature of resistance and alternative control options.

II.3.7 Research and educational needs

The respondents were asked to indicate their perspective on current needs of research and extension efforts for resolving weed management issues. About 67% of the respondents emphasized on developing new strategies to control herbicide-resistant weeds and rated it as one of the most important research needs. With the prevalence of herbicide-resistant weeds spreading in the US rice production, stakeholders are aware of the importance of controlling them. Therefore, development of effective strategies to delay the evolution of herbicide-resistant weeds is in high demand. Nearly 57% of the respondents indicated new herbicide-resistant rice varieties as one of the research priorities. Currently, the Clearfield® rice technology has been widely used. Recently, Provisia® rice technology with resistance to the acetylcoenzyme-A-carboxylase (ACCase)-inhibitor herbicide quizalofop-p-butyl (WSSA Group 1) is commercially available in the market (Hopkins 2018). This system is developed for the control of weedy rice and other grass weeds such as barnyardgrass that have evolved resistance to ALS-inhibitor herbicides. The stakeholders have emphasized additional herbicide-resistant traits in rice to allow for more herbicide options. Fifty-seven percent of respondents indicated exploration of more economical weed management options as one of the research needs. Other research areas selected by the stakeholders include improving weed control efficacy of current herbicides (43%), rice tolerance to herbicide and injury reduction (42%), and preventing weeds from forming soil seed bank (31%).

Overall, there is a critical need to focus research and extension efforts on developing diverse and integrated weed management strategies that are economical and sustainable in the long run. It is also important to protect currently available herbicides for long-term through judicious usage. Implementing management programs with multiple herbicide sites of action can be one of the ways to improve weed management efficacy and reduce the selection for herbicide-resistant weeds. More research and outreach are necessary in this regard.

II.4 Conclusions

This study presented the stakeholders’ perspective on problematic weeds and management practices in Texas rice production. Field survey results corroborated with some of the problematic weed species identified by the stakeholder survey. Most of the stakeholders expressed concerns about the evolution of herbicide-resistant weeds. The production practices need to be changed at the producer level to reduce the pace of herbicide resistance evolution in weed species. Findings from this survey can help to direct future research and outreach efforts for sustainable weed management in rice production systems in Texas.

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Table 1. Questions regarding weed management issues in Texas rice production

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1. Which of the following applies to you? Grower; Independent consultant; Dealer/distributor; Sales representative; Other_________
2. What is the approximate size of your rice farm? _________acres in __________ county
   If you are a consultant, what is the total rice acreage you consult for and in which counties?

3. What is the typical crop rotation following rice? (e.g., continuous rice, rice-fallow-rice, rice-fallow-fallow-rice, rice-soybean-rice, etc.). If more than one rotation is applicable for your operation, please list the % of the area for each rotation.

4. What pre-emergence herbicide(s) do you use or recommend most often (please rank 1 to 7 among the choices below, 1 as most often):
   Command___; Facet___; Newpath__; Bolero___; Prowl H20___; Sharpen___; Other (specify) _____
5. What post-emergence herbicide(s) do you use or recommend most often, (please rank 1 to 7)
   Newpath___; Facet___; Clincher___; Propanil (Stam, Duet, etc) ___; Regiment___; RiceStar____; Other (specify)_____
6. What are the five most problematic weeds in your or your growers' rice fields?
   (1) (2) (3)
   (4) (5)
7. What is the area under Clearfield™ rice that you grow or consult? ______acres;
   Please list any herbicide(s) other than ALS chemistry (Newpath, Beyond, Grasp, Regiment, Strada, or Permit) used in Clearfield™ rice (except for burndown) ___________________________
8. How many times do you scout rice fields for weeds in a year? _______;
   Please rate the level of weed infestation in your fields: Very serious; Serious; Moderate; None
9. What factors do you consider when making weed control decisions? Select all that apply.
   ☐ Economic threshold; ☐ Previous experiences; ☐ General field appearance; ☐ University
   ☐ recommendations; Dealer/distributor☐ recommendations; Other (specify) _____________
10. Do you use non-chemical weed management practices? If so, please specify the practices.

11. What are the constraints to adopting non-chemical weed management? Select all that apply.
    Limited options; Ineffective; Time consuming; Expensive; Other (specify) ______________
12. Approx. how much is typically spent on weed management in an acre of rice?
    Main crop ________$/acre; ratoon crop ________$/acre
13. What's the level of concern you have for herbicide-resistant weeds?
    High; Moderate; Low; None
14. What are the suspected herbicide-resistant weeds in your rice fields, if any? (Please try to provide the name of the weed and associated herbicide, e.g., barnyardgrass – propanil, facet)

15. Which of the following research topics do you think are important to you? Please check all that apply.

☐ Strategies to control herbicide-resistant weeds
☐ Developing new herbicide-resistant rice varieties
☐ Economical weed management practices
☐ Improve the weed control efficacy of current herbicides
☐ Rice tolerance to herbicides and injury reduction
☐ Prevent weeds from forming soil seed bank
☐ Others, please indicate _______________

Any other comments that will help direct our research and extension efforts:

Table 2. Frequency of occurrence (%) and average coverage (%) of different weed species documented during late-season field surveys in rice fields in Texas

Common name	Scientific name	Frequency of occurrence[a] (%)	Average coverage[b] (%) ± SE[c]
Junglerice	*Echinochloa colona*	65	13 ± 2
Nealley's sprangletop	*Leptochloa nealleyi*	43	3 ± 1
Hemp sesbania	*Sesbania herbacea*	31	11 ± 7
Barnyardgrass	*Echinochloa crus-galli*	18	5 ± 3
Common waterhemp	*Amaranthus tuberculatus*	13	3 ± 1
Weedy rice	*Oryza sativa*	10	11 ± 3
Northern jointvetch	*Aeschynomene virginica*	3	5 ± 3
Sedges	*Cyperus* spp.	3	2 ± 1
Texasweed	*Caperonia palustris*	3	1 ± 1.3

[a] Percentage of the surveyed fields where the species was present

[b] Average coverage (% field area infested) where the species was present

[c] SE =Standard Error of the Mean

Table 3. Ranking of the most problematic weeds in Texas Rice by stakeholders

Common name	Scientific name	Responses[a]	Points[b]	Rank
Barnyardgrass[c]	*Echinochloa* spp.	68	304	1
Sprangletops	*Leptochloa* spp.	46	163	2
Sedges	*Cyperus* spp.	44	117	3
Alligatorweed	*Alternanthera philoxeroides*	21	69	4
Weedy rice	*Oryza sativa*	15	60	5
Broadleaf signalgrass	*Brachiaria platyphylla*	22	59	6
Pigweed[d]	*Amaranthus* spp.	17	52	7
Hemp sesbania	*Sesbania herbacea*	11	40	8
Dayflower	*Commelina* spp.	9	24	9
Northern jointvetch	*Aeschynomene virginica*	7	21	10
Texasweed	*Caperonia palustris*	6	19	11
Crabgrass	*Digitaria* spp.	4	14	12
Ducksalad	*Heteranthera limosa*	3	9	13
Dallisgrass	*Paspalum dilatatum*	2	7	14
Texas millet	*Urochloa texana*	1	5	15
Johnsongrass	*Sorghum halepense*	1	4	16
Water parsley	*Oenanthe sarmentosa*	1	4	17

[a] Number of responses specified this species out of the 108 questionnaires returned

[b] Points were calculated by assigning values of 5, 4, 3, 2, and 1 to the first, second, third, fourth, and fifth most problematic weed specified by each respondent and then summing all values

[c] The majority of the species was junglerice (*E. colona*), but the respondents generally combined both *E. colona* and *E. crus-galli* (barnyardgrass)

[d] The specific pigweed species occurring in the Texas rice belt is common waterhemp (*A. tuberculatus*)

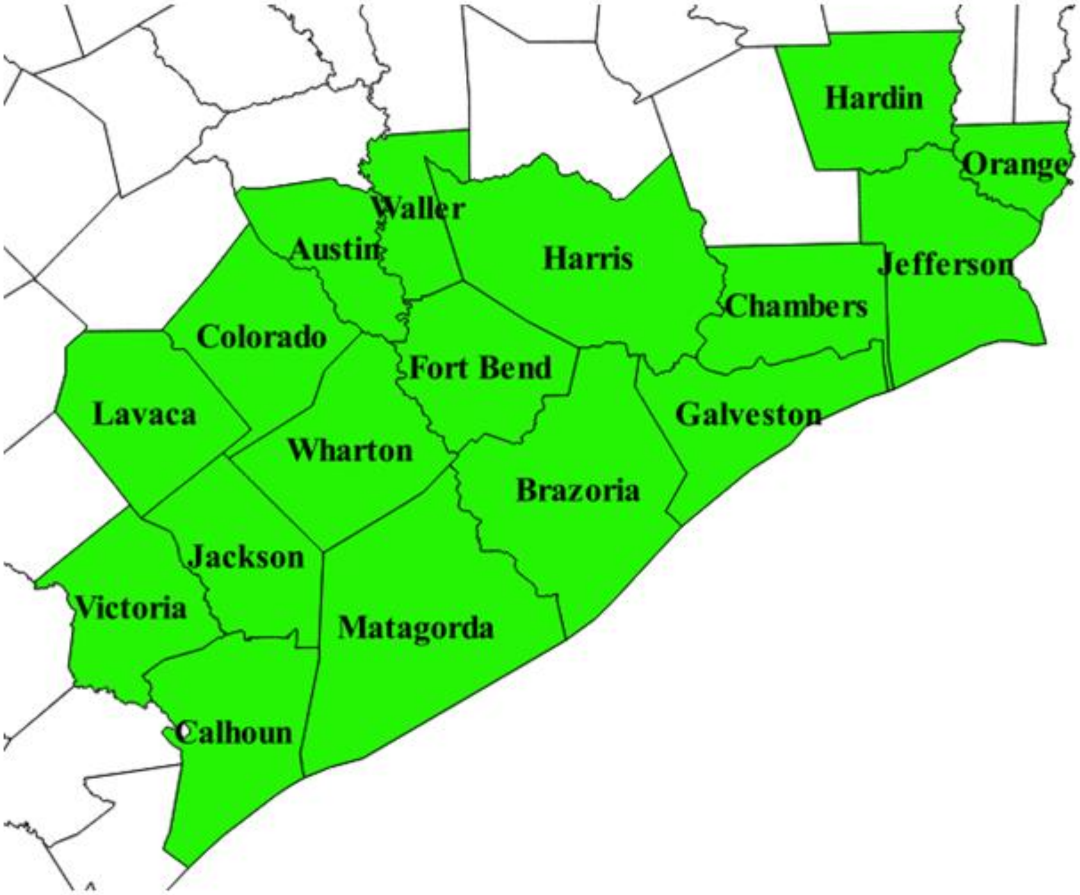

Figure 1: Historical rice growing counties in Texas

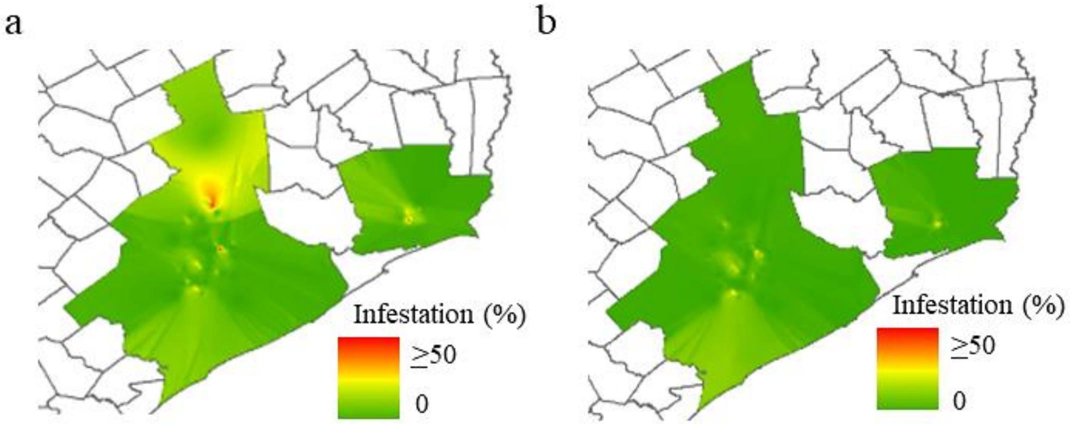

Figure 2: ArcGIS maps showing late-season field infestation by a) all weed escapes; b) *E. colona* (junglerice)

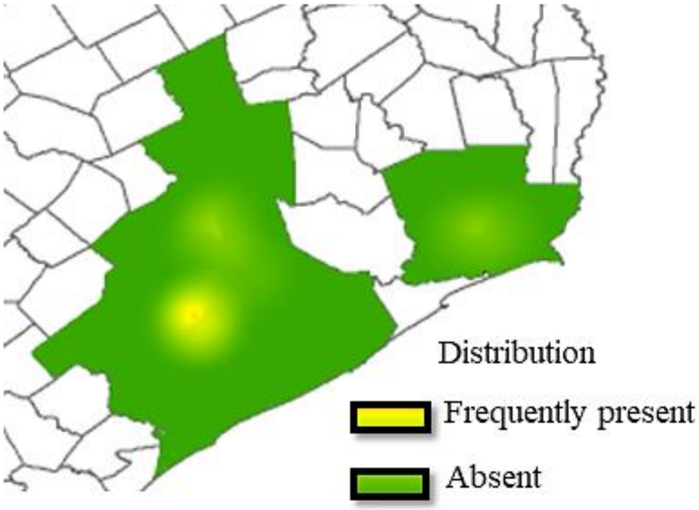

Figure 3: ArcGIS maps showing late-season field distribution of weedy rice

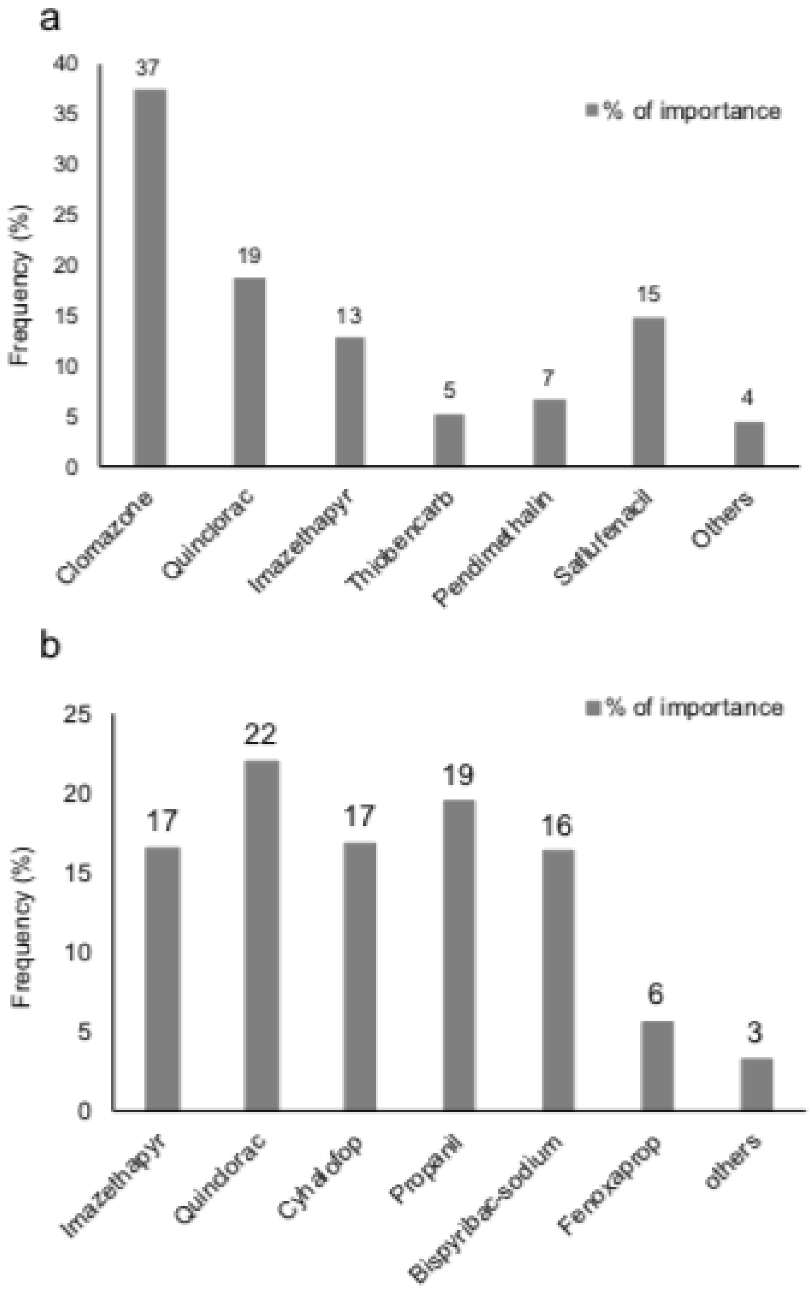

Figure 4: The importance (%) of frequently used a) Pre-emergence herbicides, including clomazone (Command®), quinclorac (Facet®), imazethapyr (Newpath®), thiobencarb (Bolero®), pendimethalin (Prowl®), saflufenacil (Sharpen®), or others; b) Post-emergence herbicides, including imazethapyr (Newpath®), quinclorac (Facet®), cyhalofop (Clincher®), propanil (Riceshot®), bispyribac-sodium (Regiment®), fenoxaprop (RiceStar®), or others. Importance (%) was calculated based on points given (7 = the most often used/recommended PRE-herbicide, and 6 = the most often used/ recommended POST-herbicide; 1 = least used/ recommended herbicide).

CHAPTER III

CONFIRMATION AND CHARACTERIZATION OF HERBICIDE RESISTANCE IN BARNYARDGRASS AND WEEDY RICE IN RICE PRODUCTION FIELDS IN TEXAS

III.1 Introduction

Weeds can cause significant yield losses by competing with crops for light, nutrition and other resources (Oerke 2006). Barnyardgrass [*Echinochloa* crus-galli (L.) P. Beauv.] and weedy rice (*Oryza sativa* L.) are two problematic weeds in rice production worldwide. For barnyardgrass, high seed production potential, seed dormancy and genetic diversity makes it adaptable to various environmental conditions (Lopez-Martinez et al. 1999; Maun and Barrett 1986). Likewise, weedy rice exhibits high seed shattering, seed dormancy, and extended seedbank persistence (Goss and Brown 1939; Noldin 1995). Both of these species can cause up to 80% yield loss in rice if not controlled adequately (Burgos et al. 2008; Diarra et al. 1985; Smith 1983).

Rice production in Texas and other southern US states is highly mechanized, and the direct-seeded system is common (dry seeded, with delayed flooding at the 5 to 6-leaf stage) (Hill et al. 1991; Rao et al. 2007). In the direct seeded system, the risk of yield loss due to weed competition is higher compared to the puddled-transplanted production system, because of the lack of flooding to suppress weed growth (Rao et al. 2007). Further, the phenology of barnyardgrass and weedy rice seedlings closely resemble rice seedlings, making it difficult to manage them selectively through cultural/mechanical approaches.

Due to these limitations, use of herbicides has been the most preferred method to control weeds in rice.

Although barnyardgrass can be selectively controlled in rice by propanil, repeated use of this herbicide over time has led to the evolution of propanil-resistant barnyardgrass biotypes. Barnyardgrass was first reported to have evolved resistance to propanil in 1990 in Arkansas (Carey et al. 1995). Subsequently, propanil-resistant barnyardgrass has been reported in Texas, Missouri and Louisiana in 1991, 1994, and 1995, respectively (Heap 2018). Quinclorac was introduced in 1992 to control propanil-resistant barnyardgrass and soon became a widely used substitute for propanil (Malik et al. 2010). However, only a few years later, barnyardgrass resistance to quinclorac was reported in Louisiana rice in 1998 (Heap 2018). Barnyardgrass resistance to more than one herbicide site of action (SOA) began to appear subsequently. The first case of multiple-resistant barnyardgrass was reported in 1999 in Arkansas, with resistance to both propanil and quinclorac (Lovelace et al. 2000). In 2000, a barnyardgrass population was reported resistant to the acetolactate synthase (ACCase)- and lipid synthesis-inhibitors in California (Fischer 2000). Multiple resistance in barnyardgrass to four SOA (ACCase-, ALS-, PSII-, and cellulose-inhibitors) was reported in 2011 from Mississippi (Heap 2018). The number of effective herbicide options available for weed control in rice has been greatly diminished because of multiple resistance cases.

Selective control of weedy rice in rice fields is generally difficult due to the genetic similarities between the two species. In conventional rice fields, weedy rice control is limited to an effective burndown herbicide application prior to planting. In the Clearfield® rice system, weedy rice could be effectively controlled by imidazolinone (IMI) herbicides such as imazethapyr and imazamox. The Clearfield® rice is an herbicide-resistant rice technology that was developed through induced mutagenesis (Tan et al. 2005). Soon after its commercialization, widespread resistance to imazethapyr has been observed in weedy rice in many Clearfield® rice fields in southern U.S. (Burgos et al. 2008; Singh et al. 2017).

Diversifying the SOA of herbicide programs used for weed management is key to delay the evolution of herbicide resistance in weed populations (Jutsum and Graham 1995; Norsworthy et al. 2012). Knowledge of current status of weed response to herbicides can provide insights for developing effective management practices. In Texas rice production, the current status of herbicide resistance in barnyardgrass and weedy rice are not known. Therefore, the current study was conducted to (1) evaluate the response of barnyardgrass populations to clomazone, fenoxaprop-ethyl, propanil, imazethapyr, and quinclorac for possible cross- and multiple resistance, and (2) evaluate the response of weedy rice populations to imazethapyr.

III.2 Materials and Methods

III.2.1 Sample collection

Field surveys were conducted during late July to early August 2015 and 2016 to collect barnyardgrass and weedy rice seed samples across the rice growing counties of Texas prior to rice harvest (Fig.5). Survey sites were randomly selected on a Google® map based on the presence of levee- like marks, using the ITN Converter software (version 1.88; Benichou Software). The software automatically generates the most efficient route from site to site. Route files were exported to a portable global positioning system (GPS) device (TomTom International), which facilitated navigation to each site. If the site was not a rice field, or there was no barnyardgrass or weedy rice present in the field, the first population found on the way to the next survey site was collected. Approximately 20 seed heads per weed species were collected from each field where weed control failure was detected. Harvested panicles were bagged and dried in a hot-air oven at 50 °C for 48 hours. Samples were hand thrashed, cleaned, and stored in plastic Ziploc® bags prior to use in herbicide assays. A total of 60 barnyardgrass samples and 10 weedy rice samples were collected.

III.2.2 Herbicide assays

Whole-plant herbicide assays were conducted at the Norman Borlaug Center for Southern Crop Improvement Greenhouse Research Facility at Texas A&M University, College Station, TX. The plants were grown at 30/26 °C day/night temperature regime and 14-hr photoperiod. For barnyardgrass, herbicide resistance evaluations were carried out for 2 pre-emergence (PRE) herbicides [clomazone (Command 3ME®, FMC Agricultural Solutions, Philadelphia, PA) and quinclorac (Facet® 75 DF, BASF corporation, Research Triangle Park, NC)] and 4 post-emergence (POST) herbicides [Propanil (RiceShot®, RiceCo LLC, Memphis, TN), Fenoxaprop (Ricestar® HT, Bayer CropScience, Research Triangle Park, NC), Imazethapyr (Newpath®, BASF corporation, Research Triangle Park, NC), and Quinclorac (Facet® 75 DF, BASF corporation, Research Triangle Park, NC)]. Weedy rice populations were evaluated for imazethapyr POST. Information about each herbicide used in the screening is provided in Table 4.

Seeds of each population were broadcast planted into 9 x 10 cm pots. For POST herbicide evaluations, commercial potting medium (Sun Gro®Sunshine®LC1 Grower Mix with RESiLIENCE™) was used. Emerged seedlings were thinned to 5 per pot at the 1- to

2-leaf stage. For PRE- herbicide evaluations, field soil without recent herbicide application history collected at the Texas A&M field research farm (30.46° N, 96.43° W) was used. Soil was kept moist for 2 to 3 weeks prior to use for PRE-assay to eliminate any pre-existed weed seeds. A non-treated control of each population was maintained for comparison. A susceptible standard control was also included for comparison. For weedy rice resistance screening, the conventional rice variety Cheniere was used as a susceptible standard. The experimental units were arranged in a completely randomized design, with 4 replications and 2 experimental runs.

Herbicide treatments were applied at the 2- to 3-leaf weed stage, using an automated spray chamber equipped with an XR8002VS nozzle (Teejet Spraying Systems), delivering 140 L/ha. Resistance was characterized based on the number of plants that survived the 1X rate (% survival) and the injury (%) response of each population compared to the non-treated standard at 21 days after application (DAA). Based on the injury levels observed, the populations were categorized as resistant (≤50% injury), less sensitive (51 to 90%) and susceptible (≥91%).

III.2.3 Dose-response assays

Dose-response assays were conducted on barnyardgrass populations that exhibited the least injury for each of the 3 POST herbicides: fenoxaprop, imazethapyr, and propanil. The seedlings were established in the greenhouse with the same procedure described in the herbicide screening experiment above. The resistant populations were treated with eight rates (0, 0.5, 1, 2, 4, 8, 16 and, 32X the labeled rate) of each of the test herbicides, whereas the susceptible populations were treated with seven rates (0, 0.0625, 0.125, 0.25, 0.5, 1, and 2X the labeled rate) of each herbicide. Four replications (5 seedlings/rep; pot size: 10

cm x 9 cm) were included for each herbicide dose and the treatments were arranged in a completely randomized design. The experiment was repeated in time. Survival and injury ratings (0 to 100%) were recorded at 21 DAA.

III.2.4 Statistical analyses

Data were subjected to ANOVA using the Statistical Analysis Software (SAS v.9.4, SAS Institute Inc., Cary NC, USA). Data were pooled across the treatments due to a lack of treatment by run interactions. Dose-response curves were developed using SigmaPlot v.13 (Systat Software, Inc., San Jose, CA). Dose-response based on the survival data were fitted to a three-parameter log-logistic equation (Eqn 1), where c is the asymptote, b is the inflection point and a is growth rate.

$$Y = c/ [1+e^{\{-a\,(x-b)\}}] \qquad [1]$$

The effective dose of herbicide that would cause 50% control (ED50) was estimated from the regression equations. Resistance ratios (R/S) were computed from their respective ED50 values divided by the ED50 of the susceptible sample.

III.3 Results and Discussion

III.3.1 Distribution of barnyardgrass and weedy rice in Texas rice production

The distribution of barnyardgrass across the Texas rice belt is shown in Fig.5. The majority of the barnyardgrass populations were collected from Lavaca, Colorado, Jackson, Wharton, Chambers, and Jefferson counties. Historically, these are the prominent rice growing counties in Texas (Yang et al. 2017). Barnyardgrass flourishes well in warm temperature conditions and can grow in a wide range of habitats, including a range of sandy and clay soils (Brown 2006; Crenwelge 2006). In Texas rice production, two different soil types (sandy loam and clay) are predominant and barnyardgrass is commonly found in both soil types. The majority of weedy rice was found in Wharton, Chambers and Jefferson counties. Overall, the distribution of weedy rice was not as widespread as barnyardgrass.

III.3.2 Weedy rice response to imazethapyr

Eleven weedy rice populations were evaluated for response to imazethapyr; all these populations survived the 1X rate of this herbicide (Table 5). Results suggest that these weedy rice biotypes escaped control measures because of resistance to ALS-inhibitors. Weedy rice is difficult to control because of its similarity with rice crop, both morphologically and genetically. With the introduction of the Clearfield® rice technology, the control of weedy rice and other weeds had been improved significantly. However, strong selection exerted by these ALS-inhibiting herbicides has led to the evolution of ALS-inhibitor resistant weedy rice populations in rice fields. The problem of ALS-resistant weedy rice has been aggravated by pollen mediated-gene flow from Clearfield® rice to weedy rice (Singh et al. 2017; Shivrain et al. 2009). Synchronization of flowering between rice cultivars and weedy rice increases the potential of outcrossing, resulting in reduced effectiveness of Clearfield® rice technology in controlling weedy rice (Shivrain et al. 2009; Gealy et al. 2003).

III.3.3 Echinochloa spp. *response to clomazone*

None of the *Echinochloa* spp. populations tested in this study showed resistance to the recommended label rate (1X) of clomazone (Table 5). This herbicide was originally introduced as an alternative herbicide to control propanil- and quinclorac- resistant barnyardgrass populations (Talbert and Burgos 2007). It is often applied along with other

POST herbicides in weed management programs to achieve low-cost annual grass control, and to extend weed control spectrum (Zhang et al. 2005, Willingham et al. 2008). Clomazone is listed as the most commonly recommended PRE-herbicide by the consultants in Arkansas (Norsworthy et al. 2013) and Texas (Liu, unpublished). As a pre-emergence herbicide, it provides excellent control of grass weeds such as *Echinochloa* spp., *Urochloa* spp., and *Leptochloa* spp. However, *Echinochloa* spp. resistance to clomazone was confirmed in Arkansas rice production in 2008 (Norsworthy 2008). Rouse et al. (2018) also reported that about 2% of the 450 accessions of *Echinochloa* spp. collected in Arkansas during 2006 to 2016 were resistant to clomazone. Even though resistance to clomazone has not been found among the *Echinochloa* spp. populations in Texas, the risk of herbicide resistance should not be neglected when making a weed control decision.

III.3.4 Echinochloa spp. *response to quinclorac*

Results of the current study showed that 62% of the 60 *Echinochloa* populations were less sensitive (51-90% injury) to quinclorac PRE (Table 5). When quinclorac was applied POST, 45% of the barnyardgrass populations were found highly resistant (0-50% injury), whereas 32% of them were less sensitive (51-90% injury). Previously, quinclorac was known to provide excellent control of propanil-resistant barnyardgrass; however, reports of resistance to quinclorac soon became common after the first case of resistance to this herbicide from Louisiana in 1998 (Heap 2018). In the following year, a barnyardgrass population collected in Arkansas was found resistant to both quinclorac and propanil at 16X the recommended rate (Lovelace et al. 2003). Quinclorac resistance was ranked the second most common problem in *Echinochloa* spp. after propanil resistance in Arkansas rice (Rouse et al. 2018). Among the 450 populations of *Echinochloa* spp. collected from Arkansas during 2006 to 2016, 23% of the populations were found resistant to quinclorac (Rouse et al. 2018). This indicates that resistance to quinclorac in *Echinochloa* spp. is widespread in rice growing regions in the U.S.

III.3.5 Echinochloa spp. *response to fenoxaprop*

Of the total 60 barnyardgrass populations evaluated, 7% of the populations were resistant (0-50% injury), 30% were less sensitive (51-90% injury), and 63% were susceptible (91-100% injury) to fenoxaprop. Resistance to fenoxaprop is the least commonly detected resistance in the *Echinochloa* spp. populations tested in this study. Till date, fenoxaprop resistance in *Echinochloa* spp. has been reported in Latin American and Asian Countries, including Bolivia, Costa Rica, Nicaragua, China, and South Korea (Heap 2018). In the U.S., multiple resistance to fenoxaprop and other herbicides in *Echinochloa* spp. has been reported in California and Arkansas (Fischer et al. 2000, Norsworthy 2013). The results of the current study indicate that fenoxaprop resistance within the *Echinochloa* spp. populations is at an early stage of development (Fig.6), because the survival levels are very low. It has been observed that survivors at an early stage of herbicide resistance evolution within a weed population are frequently neglected by the growers/consultants. The survivors are usually mistaken as individuals that escaped herbicide applications because of environmental and application factors.

The ED_{50} values determined based on the dose-response assays for fenoxaprop-resistant and -susceptible populations were 131.8 and 43 g ai ha^{-1}, respectively (Table 6). The R/S values derived from the ED_{50} values of resistant and susceptible populations have indicated that the resistant population was 3-fold more resistant to fenoxaprop compared with the susceptible standard (Table 6, Fig.7a). A population of *Echinochloa* phllopogon from California was confirmed to have resistance to fenoxaprop (ED_{50}, 110g of ai ha^{-1}), which was 10-fold less sensitive to this herbicide compared to the susceptible standard (ED_{50}, 11g of ai ha^{-1}) (Bakkali et al. 2007). Despite the low level of resistance in some areas of Texas, fenoxaprop is still considered an effective herbicide for selective grass control in rice production as rice is naturally tolerant to fenoxaprop (Stoltenberg 1989). Fenoxaprop is often mixed with herbicides that control sedges or broadleaf weeds to broaden the weed control spectrum and save time as well as application cost.

III.3.6 Echinochloa spp. *response to imazethapyr*

The high level of survival of *Echinochloa* spp. to imazethapyr indicates that resistance to this herbicide is emerging in this region (Fig.6). Of the total 50 *Echinochloa* spp. populations evaluated for imazethapyr in the current study, 60% were resistant (0-50% injury), 28% were less sensitive (51-90% injury), and only 12% were susceptible (91-100% injury). The evolution of imazethapyr resistance has coincided with the widespread use of the Clearfield^{TM} rice technology. There was a 5% declination of Clearfield^{TM} rice production observed each year in Arkansas after 2011, when the peak of 70% production occurred (Hardke 2016). Less than 10% of the *Echinochloa* spp. tested in Arkansas were confirmed to be resistant to one or more ALS-inhibitor herbicides before 2011, but from 2013 to 2016, the frequency of resistant populations increased by more than 20% (Rouse et al. 2018). The cross resistance of *Echinochloa* spp. populations to ALS-inhibitor herbicides in Arkansas was first reported in 2012 (Riar et al. 2012). Recently, Rouse et al. (2018) reported resistance to ALS-inhibitors in 114 accessions across 20 counties of Arkansas.

Dose-response regression curves could not indicate ED_{50} values for the barnyardgrass population that was highly resistant to imazethapyr, as control was <50% even at the highest rate tested (Fig.7). The ED_{50} value of the imezathapyr-susceptible population was 48 g ai ha^{-1} (Fig.8, Table 6). The R/S ratio (highest rate tested for resistant versus susceptible populations) has revealed that the resistant population was 70-fold more resistant to imazethapyr compared with the susceptible standard (Table 6). A similar case was reported in four populations of *Echinochloa* from Italy, which were confirmed to have cross-resistance to two ALS-inhibitor herbicides, penoxsulam and imazamox. The highest herbicide dose applied didn't provide 50% control compared to the untreated standard. The R/S ratio of LD_{50} for penoxsulam was >25, and for imazamox, it was >56 (Panozzo et al. 2013). The observed high resistance level indicates that target-site mutation could be the mechanism for ALS-inhibitor resistance in these populations.

III.3.7 Echinochloa spp. *response to propanil*

Propanil has been one of the most effective grass herbicides in rice production for almost 30 years since its commercialization (Smith and Hill 1990). It is still used in rice production, but its effectiveness has drastically decreased because of the evolution of propanil-resistance in weeds such as barnyardgrass. Of the total 52 barnyardgrass populations evaluated in the current study, all were resistant (0-50% injury) to propanil. These results are similar to the findings in Arkansas (Rouse et al. 2018). In a weed management survey conducted in Arkansas in 2013, 58% of the respondents listed propanil-resistant barnyardgrass as the most common problem in rice fields (Norsworthy et al. 2013). Because of the long history of propanil use in Arkansas, propanil resistance occurred at a high frequency, with 57% of the 450 total populations collected during 2006-2016 showed resistance to propanil.

The ED_{50} value of this resistant population could not be calculated as 50% control was not observed even at the highest rate tested (Fig.7). The ED_{50} values of the propanil-susceptible population was 2,428 g ai/ha (Table 6). The R/S ratio of highest rate of resistant population and ED_{50} of susceptible populations indicated that resistant population was more than 14.8-fold resistant than the susceptible population (Table 6, Fig.7). The survival range of *Echinochloa* spp. (Fig.6) indicates that resistance to propanil is already at an advanced stage and widespread throughout the Texas rice bet. This is consistent with the report received from some growers, indicating that propanil does not provide satisfactory control of grass weeds (Liu, unpublished).

III.3.8 Echinochloa spp. *resistance to multiple/ single herbicide site of action*

Multiple resistance to more than one herbicide SOA was documented in this study (Table 7), with 60% of the populations resistant to more than one herbicide SOA. Among the 60 *Echinochloa* spp. populations, 5% had resistance to all the 4 POST herbicides evaluated. Fifteen percent of the populations had multiple resistance to propanil, imazethapyr and quinclorac. Forty percent of them had multiple resistance to 2 herbicide sites of action, with 23% resistant to propanil and imazethapyr, 15% resistant to propanil and quinclorac, and 2% resistant to both propanil and fenoxaprop. The rest of the populations were resistant to one herbicide site of action, with 28% resistant to propanil, 7% to imazethapyr, and 5% to quinclorac.

Echinochloa spp. populations with multiple resistance to propanil and quinclorac were confirmed in 2010 (Malik et al. 2010). The samples were collected from farms where propanil had been used for more than 20 years, and quinclorac had been used for more than 5 years. Resistance to propanil was documented in the early 1990s (Carey et al. 1995), which was caused by the enhanced detoxification by enzyme arylacylamidase (Carey et al. 1997). When growers started to use quinclorac, propanil resistance had already existed in many of the *Echinochloa* populations. This indicated that multiple resistance to propanil and quinclorac were caused by NTSR mechanisms. Current results also showed the widespread occurrence of barnyardgrass resistance to propanil.

III.4 Conclusions

This study has indicated that herbicide resistance is prevalent in Texas rice production. All of the weedy rice populations collected were resistant to imazethapyr. The *Echinochloa* spp. populations exhibited different levels of resistance to the commonly used herbicides, imazethapyr, propanil, quinclorac, and fenoxaprop. Several *Echinochloa* spp. populations also were found to have resistance to multiple herbicide site of action. Management practices, such as using alternative herbicide modes of action and crop rotation, need to be adopted in order to slow down the evolution of herbicide resistance in weed populations.

III.5 References

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Table 4. Details of the herbicides used in herbicide resistance evaluations

Common name	**Trade name**	**Site of Action (Group)**[a]	**Rate (g ai/ae ha $^{-1}$)**	**Adjuvant**[b]	**Manufacturer**
Clomazone (PRE)	Command® 3ME	Carotenoid biosynthesis Inhibitor (13)	897	None	FMC Corporation, Philadelphia, PA
Quinclorac (PRE)	Facet® 75 DF	Synthetic auxin (4)	751	None	BASF corporation, Research Triangle Park, NC
Quinclorac	Facet® 75 DF	Synthetic auxin (4)	560	1% v/v MSO	BASF corporation, Research Triangle Park, NC
Propanil	RiceShot®	PSII-inhibitor (7)	4484	None	RiceCo LLC, Memphis, TN
Fenoxaprop	Ricestar® HT	ACCase-inhibitor (1)	86	None	Bayer CropScience, Research Triangle Park, NC
Imazethapyr	Newpath®	ALS-inhibitor (2)	105	1% v/v COC	BASF corporation, Research Triangle Park, NC

[a] Abbreviations: PSII, photosystem II; ACCase, Acytle CoA Carboxylase; ALS, Acetolactate Synthase
[b] MSO- Methylated seed oil; COC- Crop oil concentrate

Table 5. Herbicide resistance profile evaluated in barnyardgrass samples collected from Texas rice production

Herbicide	Application Timing	Resistant[a]	Less sensitive[a]	Susceptible[a]	Total[b]
		--------- % of populations ---------			
Clomazone	PRE	0	0	100	60
Quinclorac	PRE	0	62	38	60
Quinclorac	POST	45	32	23	56
Fenoxaprop	POST	07	30	63	60
Imazethapyr	POST	60	28	12	50
Imazethapyr	POST	100	0	11	11[c]
Propanil	POST	100	00	00	52

[a] Resistant- 0 to 50% injury, less sensitive- 51- 90% injury, and susceptible- 91 to 100% injury

[b] Total number of populations evaluated for each herbicide

[c] Weedy rice populations

Table 6. LD_{50}[a] values and resistance ratios for the highest resistant barnyardgrass populations sampled in Texas rice production

Herbicide	Population	LD_{50} (g ai ha^{-1})	SE	R^2	RMSE	R/S[b]
Fenoxaprop	R	131.8	7.4	0.94	9.3	3
	S	43	-	0.98	7.2	
Imazethapyr	R	-	-	-	-	>70[c]
	S	48	1	0.99	4	
Propanil	R	-	-	-	-	>14.8[d]
	S	2428	185	0.92	10.7	

[a] LD_{50} is the herbicide rate that could cause 50% plant mortality at 21 days after herbicide application

[b] R/S is resistance ratio, which was derived based on the LD_{50} values of the resistant population relative to the susceptible standard

[c, d] The R/S ration could not be developed on imazethapyr and propanil resistant populations, because complete mortality wasn't able to be achieved at the highest rate(32X)

Table 7. Resistance to multiple/single herbicide site of action in barnyardgrass populations collected from Texas rice fields

No. of SOA[a]	Herbicides	% of R[b] populations
4	propanil x imazethapyr x quinclorac x fenoxaprop	5
3	propanil x imazethapyr x quinclorac	15
2	propanil x imazethapyr	23
2	propanil x quinclorac	15
2	propanil x fenoxaprop	2
1	propanil	28
1	imazethapyr	7
1	quinclorac	5

[a] SOA= site of action

[b] R= resistant

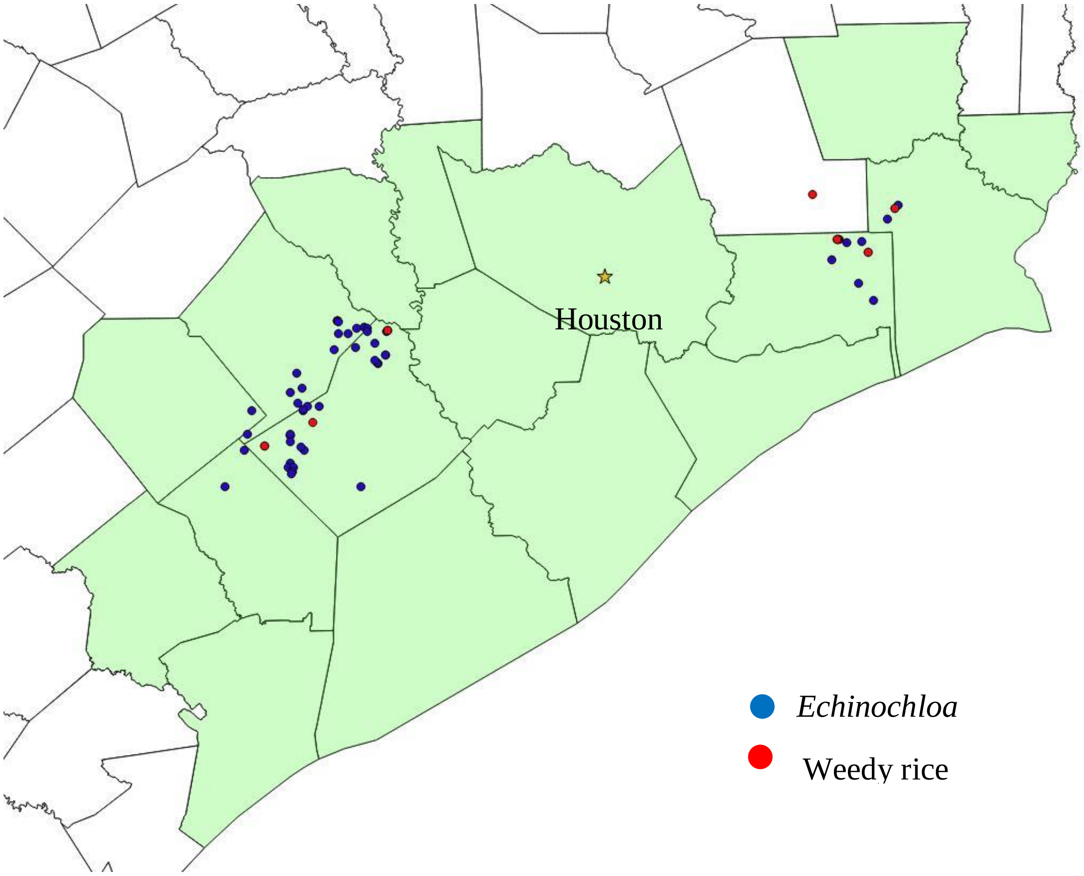

Figure 5: *Echinochloa* spp. and weedy rice sampling sites across Texas rice producing areas

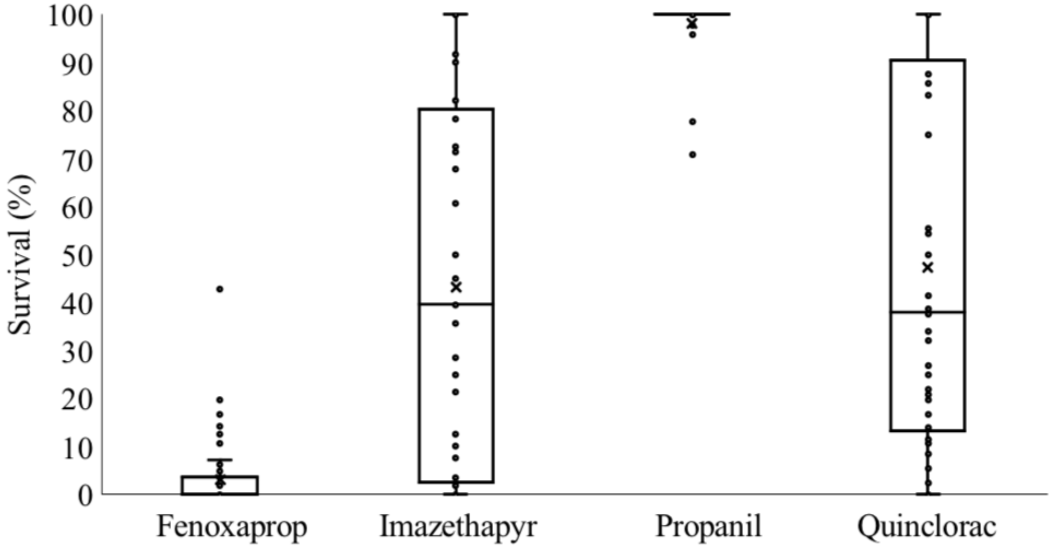

Figure 6: Survival frequency of *Echinochloa* spp. to POST herbicides, fenoxaprop, imazethapyr, propanil, and quinclorac. Frequency of survival indicates the advancement stage of resistance in a given field, for example, if the frequency of survival is 50%, it means that half of the individuals in the population has built resistance to the herbicide, and the resistance is highly noticeable in the production field.

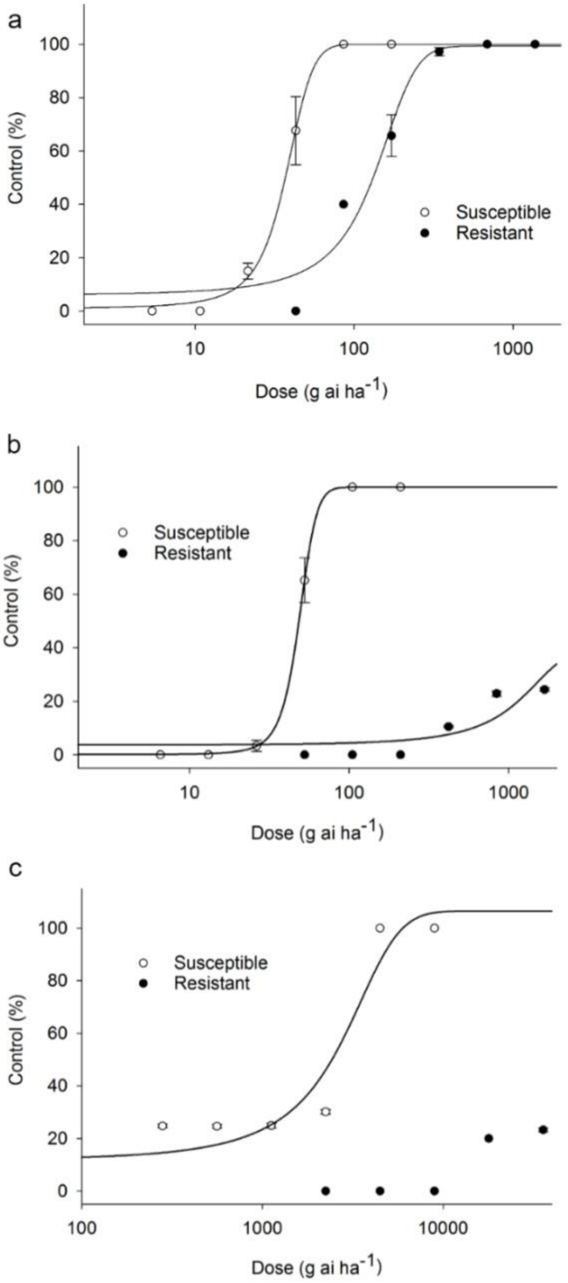

Figure 7: Dose- response analyses of resistant and susceptible *Echinochloa* spp. populations to a) fenoxaprop; b) imazethapyr; c) propanil. Recommended field rates used for fenoxaprop= 86 g ai/ha, imazethapyr= 105 g ai/ha; propanil = 4484 g ai/ha.

CHAPTER IV

PHENOTYPIC CHARACTERIZATION OF *ECHINOCHLOA* SPECIES

IV.1 Introduction

The genus *Echinochloa* contains the most troublesome weeds of rice production systems worldwide. It comprises about 50 sub-species that are most commonly found in tropical and warm regions (Michael 1983). The species in the genus *Echinochloa* are highly adaptive and competitive due to their weedy traits such as high seed production potential, seed dormancy, and genetic diversity (Lopez-Martinez et al. 1999; Maun and Barrett 1986). Barnyardgrass (*E. crus-galli*) and junglerice (*E. colona*) are the two most problematic subspecies in the southern U.S. rice production (Bryson and Reddy 2012). Barnyardgrass is an annual grass weed that looks very similar to rice at the seedling stage and can be found in crop fields or along field edges and roadsides. However, by the time it can be easily differentiated from rice, crop yield loss may already have occurred (Holm et al. 1977). It is an erect growing plant and can grow up to 2 m tall. The leaves of barnyardgrass are linear to lanceolate which can grow up to 40 cm in length, and 0.5–1.5 cm in width (Chin 2001). Junglerice, similar to barnyardgrass in appearance, is an annual grass weed. It has a prostrate growth habit and can grow up to 0.6 m in height (Hruševar et al. 2015). In general, junglerice has lanceolate shaped leaves with green or purplish stems and often branched at the base (Zimdahl et al. 1989). Rough barnyardgrass [*E. muricata* (P. Beauv.) Fernald] is another species found in U.S. rice production. It has diverse phenotypic characteristics compared to other *Echinochloa* spp. mentioned earlier. It is often erect and grows about 0.8 to 1.6 m tall. The stem nodes are hairless or have sparse hairs on it (Gould et al. 1972). Junglerice and barnyardgrass can flower throughout the year, while rough barnyardgrass usually flowers in mid-summer to early fall (Chauhan and Johnson 2009; Tahir 2016; Vengris et al., 1966).

The taxonomy of the genus *Echinochloa* is complex due to continuous morphological variation exhibited by the taxa and the formation of several intergrading polymorphic complexes which are difficult to classify (Barrett and Wilson 1981). In many cases, species of this genus lack conspicuous identification characters, which leads to misidentification (Costea and Tardif 2002; Michael 1983). Although *Echinochloa* species show a high degree of autogamy, occasional outcrossing is sufficient to causegene exchange among populations (Maun and Barrett 1986). In rice production in Texas, *Echinochloa* crus-galli was considered the most prominent species of *Echinochloa* (personal communication with growers and consultants). However, recent surveys of rice fields in Texas have suggested the occurrence of more *E. colona* populations than previously believed (Liu, personal observation).

In U.S. rice production, different *Echinochloa* spp. were reported to have evolved resistance to various herbicides (Heap 2018). For example, barnyardgrass was first found resistant to propanil in 1990 in Arkansas (Carey et al. 1995). Subsequently, barnyardgrass resistance to propanil was observed in Texas, Missouri and Louisiana in less than 5 years (Heap 2018). Quinclorac was introduced in 1992 mainly for controlling propanil-resistant barnyardgrass populations and soon it was widely used throughout the southern U.S. rice production (Malik et al. 2010). However, in 1998, barnyardgrass resistance to quinclorac was reported in Louisiana rice (Heap 2018). Multiple herbicide resistance has also been reported in *Echinochloa* spp. throughout the mid-southern U.S. rice production (Rouse et al. 2018). In Texas, multiple resistance in *Echinochloa* spp. was confirmed for propanil, quinclorac, imazethapyr and fenoxaprop (Liu, unpublished).

Though *Echinochloa* spp. are important weeds in rice production in Texas, knowledge on the occurrence, distribution and characteristics of different species and their ecotypes is limited. Moreover, it is unclear whether there is any association between phenotypic characteristics and sensitivity to herbicides in *Echinochloa* spp. An understanding of these aspects can assist in developing effetive weed management strategies. The objective of this research was to characterize different *Echinochloa* ecotypes collected from rice production fields in Texas using phenological traits, and determine potential association between phenotypic traits and herbicide resistance.

IV.2 Materials and Methods

IV.2.1 Plant materials

Field surveys were conducted during late July/early August in 2015 and 2016 to collect seed samples from barnyardgrass escapes prior to rice harvest across the Texas rice belt, primarily the areas west and east of Houston (Fig. 8). Survey sites were randomly selected on a Google® map without any prior knowledge of the distribution of *Echinochloa* spp. in specific fields, using the ITN Converter software (version 1.88; Benichou Software). The rice production areas were identified in the Google® map based on the presence of levee-like structures. This software also optimizes the most efficient travel route between the survey sites. The itinerary files were exported to a portable global positioning system (GPS) device (TomTom International), which facilitated the navigation to each survey site. If the site was not a rice field, or there was no *Echinochloa* spp. present in the field, the first ecotype found on the way to the next survey site was collected. In each field, approximately 20 panicles were randomly collected and pooled into a single sample. These are typically the individuals that escaped weed control measures implemented during the growing season. A total of 54 *Echinochloa* ecotypes were collected. The samples were subsequently dried in a hot-air oven at 50 °C for 48 hours, hand thrashed, cleaned, and stored in Ziploc® bags prior to use in the experiment. Sensitivity of these ecotypes to different herbicides (propanil, quinclorac, imazethapyr, and fenoxaprop) were evaluated in a parallel experiment described above.

IV.2.2 Morphological characterization

Seedlings of each ecotype were raised at the Norman Borlaug Center for Southern Crop Improvement Greenhouse Research Facility at Texas A&M University during March, 2017. Seeds were germinated in petridish (d = 9 cm) and transplanted to individual pots (10 x 9 cm) at the 1-leaf stage. When the seedlings reached about 15 to 25 cm tall, they were transplanted in a common garden under field conditions at the David Wintermann Rice Research Station near Eagle Lake, TX. The experiment included the 54 *Echinochloa* ecotypes established in rows (7 plants/row), arranged in a randomized complete block design with four replications (i.e. four rows of 7 plants each per ecotype). The experimental area was maintained weed-free through a combination of hand-weeding and application of glyphosate between rows using a sponge roller. The plots were surface irrigated as needed. In each row, three plants were randomly selected and tagged for detailed morpho-physiological characterization.

A total of 13 traits were recorded from each tagged plant, including stem angle; stem color; leaf color; leaf texture; flag leaf length, width, and angle; days to flowering;

panicle length; plant biomass; seed shattering; fecundity; and seed dormancy. Growth habit was determined visually relative to the horizontal plane (1 = prostrate/open canopy, 2 = intermediate 3 = erect/closed canopy). Flag leaf angle was determined visually relative to the main culm (1 = leaf angle <45°; 2 = leaf angle >45°). The length and width (measured at the widest point of the leaf) of flag leaves were measured from five tillers per plant. Leaf texture was evaluated by rubbing the finger along the leaf surface (1 = smooth; 2 = intermediate; and 3 = rough). Plant height was measured from the base of the plant to the tip of the panicle on the main stem. Plants were monitored weekly for initiation of flowering. The length of five random fully developed panicles were measured for each plant. After morphological characterization, entire plants were enclosed in Delnet® bags (Delstar Technologies, Middletown, DE, USA) to collect the shattered seeds. Visual seed shattering (%) of each plant was recorded before bagging. Plants were harvested at maturity by clipping the entire plant from the base. Samples were dried and weighted for determination of plant biomass. Seeds were thrashed and weighted for estimation of fecundity. Taxonomical classification of each of the *Echinochloa* ecotype was carried out based on Carretero (1981).

IV.2.3 Evaluation of seed dormancy

Seed samples were stored at room temperature after harvest. Seed germination tests were carried out for all *Echinochloa* ecotypes at 210 d after harvest. Twenty-five seeds of each ecotype were placed in a Petri-dish (9 cm diameter) with moistened filter paper and incubated at 30°C. The test included two replications and two experimental runs for each ecotype. Petri-dishes were arranged in a completely randomized design. Germinated seeds were counted and removed every 4th day, up-to 12 days. The Petri-plates were re-randomized after each evaluation period. Viability of non-germinated seeds were determined using the tetrazolium seed viability test (Overaa 1984). Seed were immersed in 2, 3, 5-triphenyltetrazolium chloride (1%) staining solution and incubated at 30° C for 24 hours. Seeds with pink or red embryos were considered alive and dormant.

IV.2.4 Data analysis

Statistical analyses were performed using JMP® Pro (v.13.1). All data were subjected to analysis of variance (ANOVA). The differences in quantitative traits among the ecotypes were tested using one-way ANOVA and means were separated using the Fisher's protected LSD test ($\alpha = 0.05$). A principal component analysis (PCA) was conducted using JMP® Pro (v.13.1) based on the 13 morpho-physiological variables to determine the most important traits that contribute to grouping of ecotypes using "Eigen" values. From PCA results, 4 variables were chosen as principal traits for K-means clustering to group the *Echinochloa* ecotypes. The number of clusters were determined by fit statistic, with the largest CCC (Cubic Clustering Criterion) value, using JMP® Pro (v.13.1).

IV.3 Results and Discussion

IV.3.1 Species composition

Of the total 54 ecotypes characterized, 52 were identified as *E. colona* and one each was *E. crus-galli* and *E. muricata* (Table 8), based on the taxonomic characteristics of the plants. Results show that *E. colona* is the most dominant *Echinochloa* species in rice production in Texas. Similar findings have been reported by Bryson and Reddy (2012) in a study involving 240 *Echinochloa* seed samples collected from 6 midsouthern U.S. states (Alabama, Arkansas, Kentucky, Louisiana, Mississippi, and Tennessee), where *E. colona* was the most commonly found species throughout the region. Likewise in eastern Arkansas, 73 of the 94 accessions of *Echinochloa* collected from rice and soybean fields were identified as *E. colona*. The rest of the samples included *E. crus-galli, E. muricata* and *E. walteri* (coast cockspurgrass). In the present survey, *E. walteri* was not observed in rice fields in Texas. *E. colona* had the highest fecundity and lowest seed dormancy compared to the other three species, and these two traits have likely contributed to the widespread occurrence of this species in the southern U.S. (Tahir 2016).

IV.3.2 Phenotypic differentiation within and among Echinochloa species

About half (48%) of the *E. colona* ecotypes showed prostrate growth habit, while the rest were intermediate (Table 9). Both *E. crus-galli* and *E. muricata* were erect. Prostrate growth habit allows a plant to have maximum ground coverage with open canopy, whereas an erect growth habit with closed canopy helps avoid shading from nearby plants. Moreover, the plants of *E. colona* were shorter (~48 cm) compared to those of *E. crus-galli* (127 cm) and *E. muricata* (137 cm) (Table 9), which corroborates the observations of Tahir (2016) in Echinochloa ecotypes characterized in Arkansas.

The majority (73%) of *E. colona* ecotypes had green leaf color, while the rest (27%) had plants with mix of purple and green leaf colors within the same ecotype (Table 9). *E. muricata* and *E. crus-galli* had leaf colors ranging from purple to green. It is likely that ecotypes showing a mixture of phenotypic traits might have resulted from interspecific hybridization in the recent past (Singh et al. 2017; Burgos et al. 2014). Tahir (2016) indicated that *E. muricata* produced green leaves with purple margins, which may help distinguish this species from *E. crus-galli*. With respect to stem color, 92% of the *E. colona* ecotypes had purple stem, while the rest had a mixture of both purple and green stems. *E. muricata* had green-based stems, whereas *E. crus-galli* had both green- and purple-based stems.

Nearly 87% of the *E. colona* ecotypes had smooth leaf texture, whereas the rest had a mixture of individuals with both smooth (hairless) and rough textured leaves; *E. crus-galli* and *E. muricata* had smooth leaves. This finding is consistent with the report of Holm et al. (1977) in *Echinochloa* spp. collected in the Mississippi Delta region of Arkansas, where *E. colona*, *E. crus-galli* and *E. muricata* all had smooth (hairless) leaf blades. The authors did notice that *E. muricata* may have some long hairs at the base of the leaf blades (Holm et al. 1977), but we didn't find this leaf characteristic in our *E. muricata* ecotypes. The average flag leaf length of *E. colona* was 11 cm, considerably shorter than that of *E. crus-galli* (17 cm) and *E. muricata* (18 cm). Likewise, the flag leaf width of *E. colona* was narrower (8 mm) than that of *E. crus-galli* and *E. muricata* (12 cm). The surface area of a flag leaf is associated with photosynthetic capacity (Evans and Rawson 1970). It determines the amount of light intercepted by the plant, and influences plant canopy formation and biomass.

Leaf angle is generally associated with the potential for light interception (Damalas et al. 2008). The more the leaves are closer to the horizontal angle, the higher the light interception will be. In the current study, the majority (92%) of *E. colona* ecotypes had individuals with variable leaf angles (0 to 90°), whereas 4% of them had erect leaves (angles < 45°). In contrary, *E. crus-galli* and *E. colona* had leaves with wider angles (46 to 90°). Results indicates that *E. colona* is generally more efficient in harvesting light compared to *E. crus-galli* and *E. muricata*; which is beneficial for *E. colona* as they are usually short. Conversely, wider leaf angles of *E. crus-galli* and *E. muricata*, combined with tall growth stature can allow them to shade nearby plants (Weiner and Thomas 1986). Previous reports have indicated that *E. colona* and *E. crus-galli* can flower throughout the year (Chauhan and Johnson 2009; Vengris et al. 1966), whereas *E. muricata* usually flowers during mid-summer to early fall (Tahir 2016). These corroborate with our findings. The majority (95%) of *E. colona* flowered at 28 to 35 days after transplanting (DAT), except for one ecotype that flowered about 7 days earlier than all other ecotypes. Both *E. crus-galli* and *E. muricata* flowered within the same time frame as the majority of *E. colona*. *E. colona* had the shortest (9.9 cm) panicle length compared to *E. crus-galli* (17.2 cm) and *E. muricata* (17.3 cm). Long flowering window increases opportunities for outcrossing between *Echinochloa* spp. Even though they are autogamous and predominantly self-pollinating, occasional outcrossing could still happen (Maun and Barrett 1986). Pollen-mediated gene flow was detected among *E. crus-galli* plants at the maximum tested distance of 50 m (Bagavathiannan et al. 2014). This could raise more concern, given the likelihood for the spread of herbicide resistance among *Echinochloa* spp. across a landscape.

Two different panicle colors are usually observed in *Echinochloa*: purple and green. The majority (63%) of *E. colona* had a mix of both purple and green colored panicles within the ecotype (Table 9), whereas 33 or 4% of the rest of the ecotypes had purple or green colored panicles, respectively. Both *E. crus-galli* and *E. muricata* had green colored panicles. Upon maturity, the caryopsis of the green panicles typically turn brown, while the purple colored panicles turn dark brown.

Seed shattering is an important weedy trait, which contributes to species persistence. Results showed that *E. crus-galli* and *E. muricata* had greater seed shattering abilities with 30 and 43% shattering, respectively, compared to *E. colona* (20% shattering). Further, seed dormancy is a bet-hedging mechanism that allows a species persist under unpredictable environmental conditions. The freshly harvested seeds of *Echinochloa* spp. typically exhibit dormancy, the length of which can vary from 3 to 7 months (Honěk A and Martinková 1996). Seed dormancy leads to asynchronous seedling emergence and allow them to escape herbicide treatments and other weed control measures.

We tested the seed germination 7 months after harvest. The germination of the 3 species were similar, with 83% for junglerice, 85% for barnyardgrass, and 86% for rough barnyardgrass. This means that the dormancy of most seeds had already break at this time. In Texas rice production, the fallow fields are typically used for grazing by animals. After weed emergence, grazing fallow fields could help effectively manage the *Echinochloa* spp.

IV.3.3 Clustering of ecotypes based on four significant phenotypic traits

A principal component analysis (Fig. 9) indicated that four of the 13 phenological traits characterized in the study had significantly contributed to the overall morphological diversity of *Echinochloa* spp., which included plant height, flag leaf length, seed shattering, and seed germination. Based on these four traits, K-means cluster analysis grouped the 54 *Echinochloa* ecotypes into five clusters (Table 10; Fig. 11). Frequency distribution of the four traits that significantly contributed to the *Echinochloa* spp. ecotype diversity were displayed in Fig. 10.

Cluster 2 representing the highest number of ecotypes (43%) showed the highest germination capacity (88%). It also had ecotypes with purple or green colored panicles. Cluster 1 was comprised of 28% of the characterized ecotypes, with an average plant height of 51 cm, slightly taller than that of plants in Cluster 2 (47 cm), and an average flag leaf length of 12 cm. Seed shattering was the lowest (14%) in this cluster. Seed shattering is an important weedy trait which influences the persistence of a weed population (Holm et al. 1977). High seed shattering eventually leads to high levels of seedbank replenishment as opposed to being removed by the combine harvesters, though machineries can still help disperse weed seeds during harvest if not removed in the seed fraction. Conversely, weed seeds that are captured in the crop seed fraction can affect quality and contribute to dockage (Ottis and Talbert 2007). Low weed seed shattering ability of certain ecotypes can be exploited in harvest-time weed seed control strategies. This approach allows the capture and destruction of weed seeds during harvest (Walsh et al. 2017).

Cluster 3 consisted of 13% of the total ecotypes, having the shortest flag leaf length (9 cm) and the greatest level (40%) of seed shattering. The plants in this cluster were the shortest (43 cm) compared to other groups. Cluster 4 consisted of 19% of the ecotypes characterized. This cluster had the lowest germination rate (66%). This is notable because plants in other clusters showed very high germination. Viability test results show that most of the seeds which didn’t germinate were non-viable seeds. Seed dormancy allows the seeds to persist even under favorable environmental conditions (Baskin and Baskin 2004). This adds to the difficulty of predicting the germination timing of a weed and makes timely weed control more difficult. Seed dormancy is regulated by both genetic and environmental factors; for example, temperature and various requirements for after-ripening (Bewley 1997; Chauhan and Johnson 2009). Additionally, seed shattering was also low (18%).

Cluster 5 had two ecotypes that were phenotypically very distinct from others. A taxonomic identification revealed that these two populations were *E. crus-galli* and *E.*

muricata. Plants were the tallest (132 cm) with the largest flag leaf (length-18 cm; width-12 mm) and panicle length (172 mm). Taller plants can suppress the surrounding vegetation through shading and thereby can be more competitive for resources (Weiner and Thomas 1986). This cluster had the second highest seed shattering (36%) and germination capacity (86%).

IV.3.4 Correlation analysis of morpho-physiological traits and their association with herbicide resistance

Correlation analysis performed on 13 morpho-physiological traits revealed that plant height was highly correlated with flag leaf width (0.79), panicle length (0.92), stem color (0.91) and canopy structure (0.83). Plant height, leaf width and panicle lengths have been found to be highly associated in characterization of *Echinochloa* spp. (Michael 2003; Tahir 2016). In general, barnyardgrass and rough barnyardgrass ecotypes in the current study were tallest and exhibited wider flag leaves and longer panicles. Similar findings were reported by Tahir (2016), where widest flag leaves and longest panicles were associated together with rough barnyardgrass and barnyardgrass when compared with junglerice. The qualitative trait like stem color was also highly correlated with canopy structure (0.81) which governs the plant growth habit. This indicates that *Echinochloa* ecotypes with purple stem color tend to have open geometry with shorter stature. Conversely, ecotypes with greener stems had taller plants with closed canopy. Open canopy allows for better light penetration and coverage (e.g. junglerice). However, open canopy with decumbent or prostrate growth habit may have disadvantage for hybridization when growing in close vicinity with many other tall plants. In this case, barnyardgrass and rough barnyardgrass have strong hybridization potential owing to taller stature and larger inflorescence (OLA and MAFF, 2002).

The *Echinochloa* ecotypes used in the common garden study were also evaluated in a parallel study for potential resistance to two commonly used pre-emergence rice herbicides (clomazone, quinclorac) and four commonly used post- emergence rice herbicides (propanil, quinclorac, imazethapyr, and fenoxaprop-ethyl) with different SOA (data not shown). No significant association has been observed between phenotypic traits and the herbicide resistance status (Fig. 12). The multiple herbicide resistance profile of *Echinochloa* within each cluster is displayed in Fig. 11. In Cluster 1 (total 15 ecotypes), 26% of the ecotypes were susceptible to all the four herbicides, whereas 37, 11, 21 and 5% of the ecotypes were resistant to one, two, three, and four herbicide SOA, respectively. In Cluster 2 (total 23 ecotypes), about 24, 38, and 14% of the ecotypes were resistant to one, two, and three herbicide SOA, respectively, whereas the rest 24% of them were susceptible. There was no ecotype that was resistant to all four tested herbicides in this cluster. Cluster 3 consisted of three ecotypes, of which two were resistant to one SOA, and one ecotype to three SOA. In Cluster 4 which comprised of 9 ecotypes, 22% were susceptible, whereas 42% were resistant to two SOA and 11% to three SOA. All of these four clusters comprised of only junglerice ecotypes and frequency of resistance to one SOA or more was independent of cluster groupings. Similar results have been reported by Rouse et al. (2018) in a similar study conducted in Arkansas, where 40% of the junglerice ecotypes were found resistant to only one SOA and 33% of the ecotypes were resistant to two or more herbicides. Cluster 5 in the current study consisted of two ecotypes, one of them was susceptible (barnyardgrass) and the other one (rough barnyardgrass) was resistant to one SOA. In contrast to the current findings, barnyardgrass in Arkansas exhibited higher frequency of 3-way resistance (3 SOA) compared with other species (Rouse et al. 2018). However, due to low sampling size of barnyardgrass and rough barnyardgrass in current study, within species resistance frequency could not be here compared.

IV.4 Conclusions

There are mainly three *Echinochola* spp. (*E. colona*, *E. crus-galli*, *E. muricata*) that exist in Texas rice production and the phenological traits greatly vary among the three species. Among them, *E. colona* is the most commonly occurring species in rice production fields in Texas. However, all *Echinochloa* species are commonly referred as ‘barnyardgrass’. It is vital that the research and extension community is cognizant of this inaccurate attribution. Traits such as plant height, flag leaf length, seed shattering and seed germination could help classify *Echinochloa* species. *E. colona* has medium height, medium flag leaf length, lower seed shattering ability, and average germination percentage. *E. crus- galli* is taller compared to *E. colona*, with longer flag leaf, average seed shattering ability, and average germination rate. *E. muricata* is as tall as *E. crus-galli*, and has similar flag leaf length and germination rate but highest seed shattering among three species. The diversity of the *Echinochloa* spp. and plant traits may result in the different responses to various herbicides, yet our study didn’t reflect significant correlation. Overall, the presence of high phenotypic diversities among the different *Echinochloa* ecotypes characterized in this study suggest their ability for adoption to different selection agents.

IV.5 References

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Table 8: Details of the *Echinochloa* ecotypes collected across Texas rice production

Ecotype	Latitude	Longitude	Species
ECH-1	29.35	-96.61	*E. colona*
ECH-2	29.35	-96.61	*E. colona*
ECH-3	29.23	-96.64	*E. colona*
ECH-4	29.11	-96.69	*E. colona*
ECH-5	29.12	-96.70	*E. colona*
ECH-6	29.28	-96.63	*E. colona*
ECH-7	29.27	-96.56	*E. colona*
ECH-8	29.24	-96.57	*E. colona*
ECH-9	29.19	-96.48	*E. colona*
ECH-10	29.18	-96.46	*E. colona*
ECH-11	29.16	-96.47	*E. colona*
ECH-12	29.16	-96.47	*E. colona*
ECH-13	29.16	-96.47	*E. colona*
ECH-14	29.23	-96.43	*E. colona*
ECH-15	29.37	-96.38	*E. colona*
ECH-16	29.36	-96.42	*E. colona*
ECH-17	29.35	-96.43	*E. colona*
ECH-18	29.35	-96.43	*E. colona*
ECH-19	29.38	-96.45	*E. colona*
ECH-20	29.41	-96.43	*E. colona*
ECH-21	29.57	-96.29	*E. colona*
ECH-22	29.51	-96.30	*E. crus-galli*
ECH-23	29.51	-96.30	*E. colona*
ECH-24	29.55	-96.28	*E. colona*
ECH-25	29.60	-96.30	*E. colona*
ECH-26	29.81	-94.57	*E. colona*
ECH-27	29.83	-94.44	*E. colona*
ECH-28	29.97	-94.36	*E. colona*
ECH-29	29.96	-94.35	*E. colona*
ECH-30	29.63	-96.31	*E. colona*
ECH-31	29.63	-96.31	*E. colona*
ECH-32	29.63	-96.31	*E. colona*
ECH-33	29.63	-96.31	*E. muricata*
ECH-34	29.59	-96.27	*E. colona*
ECH-35	29.54	-96.32	*E. colona*
ECH-36	29.24	-96.44	*E. colona*
ECH-37	29.59	-96.31	*E. colona*
ECH-38	29.60	-96.25	*E. colona*
ECH-39	29.61	-96.22	*E. colona*
ECH-40	29.60	-96.21	*E. colona*
ECH-41	29.59	-96.21	*E. colona*
ECH-42	29.57	-96.20	*E. colona*
ECH-43	29.55	-96.19	*E. colona*

Table 8: continued

Ecotype	Latitude	Longitude	Species
ECH-44	29.56	-96.18	*E. colona*
ECH-45	29.60	-96.14	*E. colona*
ECH-46	29.60	-96.14	*E. colona*
ECH-47	30.61	-96.35	*E. colona*
ECH-48	29.50	-96.17	*E. colona*
ECH-49	29.55	-96.27	*E. colona*
ECH-50	29.42	-96.44	*E. colona*
ECH-51	29.68	-94.43	*E. colona*
ECH-52	29.73	-94.48	*E. colona*
ECH-53	29.86	-94.47	*E. colona*
ECH-54	29.86	-94.52	*E. colona*
ECH-50	29.42	-96.44	*E. colona*
ECH-51	29.68	-94.43	*E. colona*
ECH-52	29.73	-94.48	*E. colona*
ECH-53	29.86	-94.47	*E. colona*
ECH-54	29.86	-94.52	*E. colona*
ECH-50	29.42	-96.44	*E. colona*
ECH-51	29.68	-94.43	*E. colona*
ECH-52	29.73	-94.48	*E. colona*
ECH-53	29.86	-94.47	*E. colona*
ECH-54	29.86	-94.52	*E. colona*
ECH-51	29.68	-94.43	*E. colona*
ECH-52	29.73	-94.48	*E. colona*
ECH-53	29.86	-94.47	*E. colona*
ECH-54	29.86	-94.52	*E. colona*

Table 9: Morpho-physiological characteristics of different *Echinochloa* spp. collected in rice production fields in Texas

Species[a]	N	Plant height (cm)	Flag leaf length (cm)	Flag leaf width (mm)	Panicle length (mm)	Seed shattering (%)	Germination (%)	Flowering (DAT[b])			
								28	35	21-28	28-35
E. colona	52	48	11	8	99	20	83	2	7	1	42
E. crus-galli	1	127	17	12	172	30	85	0	0	0	1
E. muricata	1	137	18	12	173	43	86	0	0	0	1

Species	N	Panicle color[c]			Stem color[c]			Canopy structure		
		P	G	P & G	P	G	P & G	Prostrate	Erect	Intermediate
E. colona	52	17	2	33	48	0	4	25	0	27
E. crus-galli	1	0	1	0	0	0	1	0	1	0
E. muricata	1	0	1	0	0	1	0	0	1	0

Species	N	Leaf texture[d]			Leaf angle (°)			Leaf color[c]		
		S	R	Intermediate	0-45	46-90	Variable (0-90)	P	G	P-G
E. colona	52	45	0	7	3	1	48	0	38	14
E. crus-galli	1	1	0	0	0	1	0	0	0	1
E. muricata	1	1	0	0	0	1	0	0	0	1

[a] Species based on plant identification

[b] DAT= days after transplanting

[c] Color: P=purple, G=green, P &G= purple and green, P-G= purple to green

[d] Leaf texture: S=smooth, R=rough, S-R= smooth to rough

Table 10: K-means cluster analysis of different *Echinochloa* ecotypes based on four most discriminating traits

Cluster[a]	N	Plant height (cm)	Flag leaf length (cm)	Flag leaf width (mm)	Panicle length (mm)	Seed shattering (%)	Germination (%)	Flowering (DAT[b])			
								28	35	21-28	28-35
1	15	51	12	8	101	14	86	0	3	1	11
2	23	47	10	8	97	21	88	1	1	0	21
3	4	43	9	7	93	40	82	0	1	0	3
4	10	48	13	8	101	18	66	1	2	0	7
5	2	132	18	12	172	36	86	0	0	0	2

Cluster	N	Panicle color[c]			Stem color[c]			Canopy structure		
		P	G	P-G	P	G	P-G	Prostrate	Erect	Intermediate
1	15	4	0	11	14	0	1	7	0	8
2	23	7	2	14	22	0	1	11	0	12
3	4	0	0	4	3	0	1	1	0	3
4	10	6	0	4	9	0	1	6	0	4
5	2	0	2	0	0	1	1	0	2	0

Cluster	N	Leaf texture[d]			Leaf angle (°)			Leaf color[c]		
		S	R	Intermediate	0-45	46-90	Variable (0-90)	P	G	P-G
1	15	13	0	2	0	0	15	P	G	P-G
2	23	21	0	2	0	0	22	0	11	1
3	4	4	0	0	0	1	3	0	17	1
4	10	7	0	3	1	0	9	0	3	1
5	2	2	0	0	1	0	0	0	7	1

[a] Clusters based on plant height, flag leaf length, seed shattering, and seed germination

[b] DAT= days after transplanting

[c] Color: P=purple, G=green, P &G= purple and green, P-G= purple to green

[d] Leaf texture: S=smooth, R=rough, S-R= smooth to rough

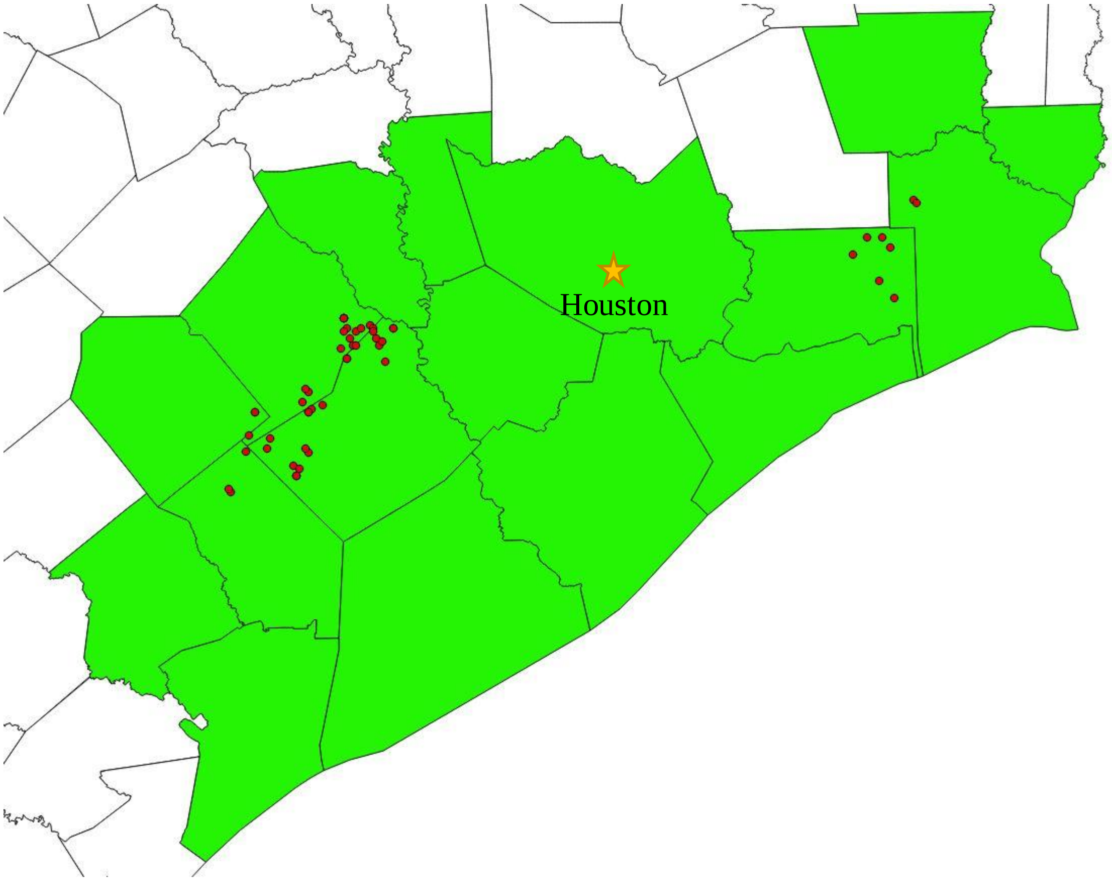

Figure 8: Collection sites of different *Echinochloa* ecotypes used in the experiment. Field surveys were conducted in all counties highlighted in green; however, *Echinochloa* escapes were only observed in the sites shown above at the time of survey prior to rice harvest.

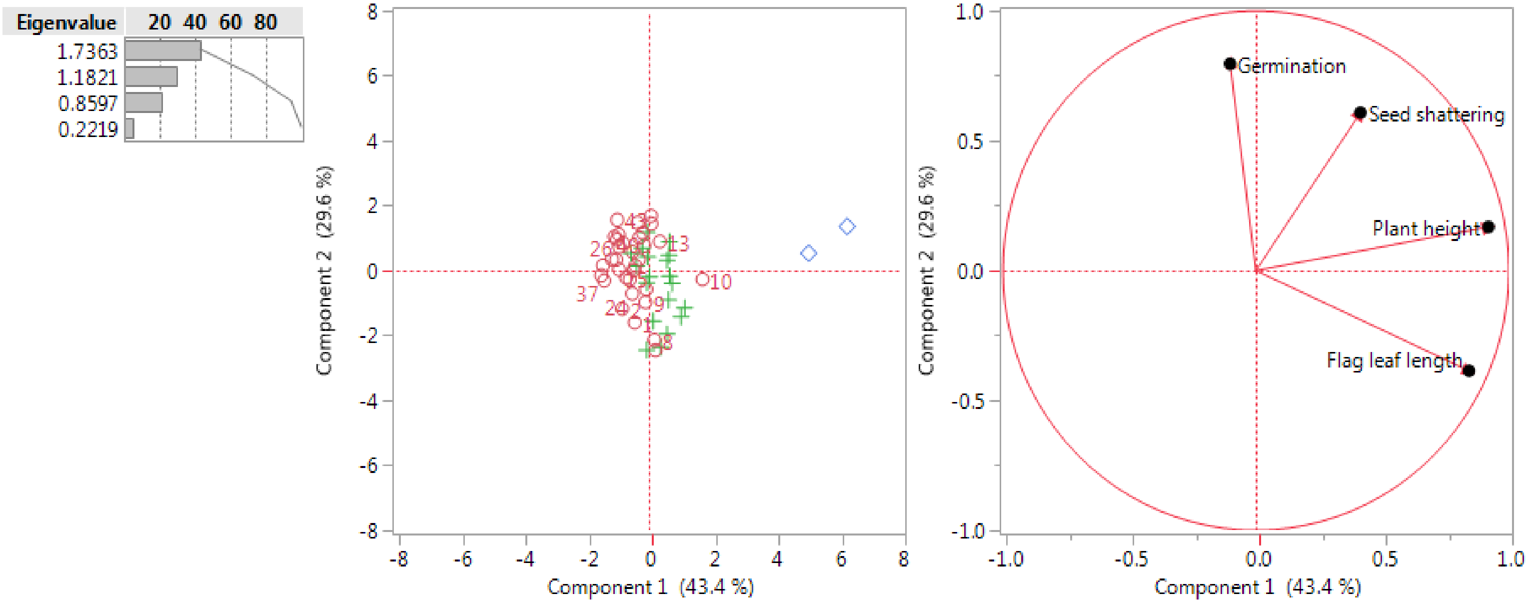

Figure 9: Principal component analysis showed four traits significantly contributed to the overall morphological diversity of *Echinochloa* spp. ecotypes. The four traits are plant height, flag leaf length, seed shattering, seed germination.

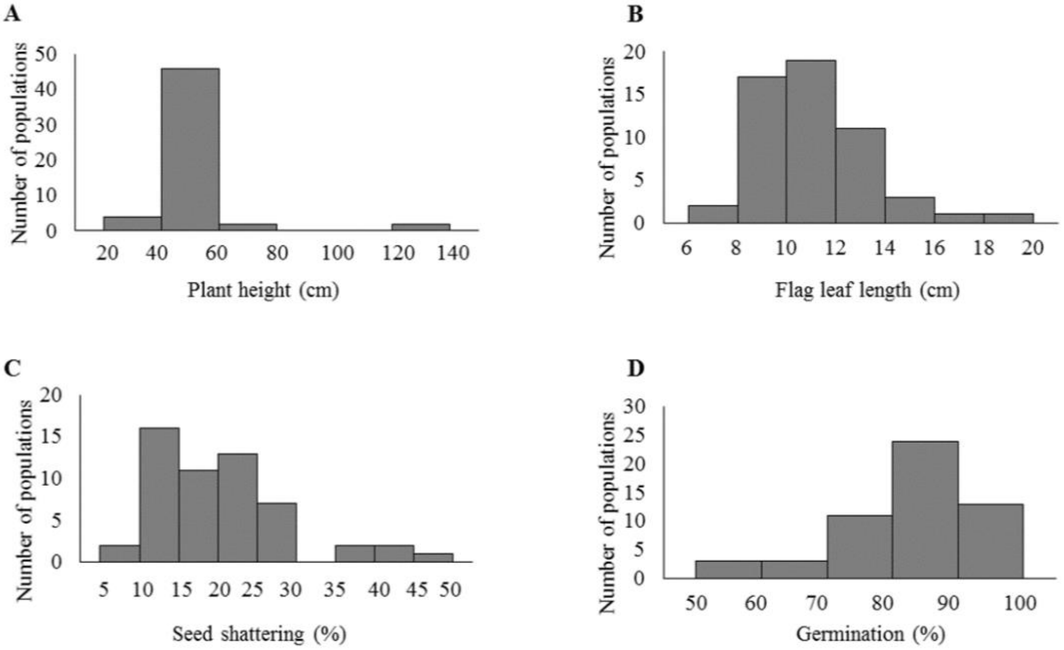

Figure 10: Frequency distribution of four *Echinochloa* spp. traits that contribute the most to principle analysis in Texas rice production. A) Plant height (cm); B) Flag leaf length (cm); C) Seed shattering (%); D) Germination (%).

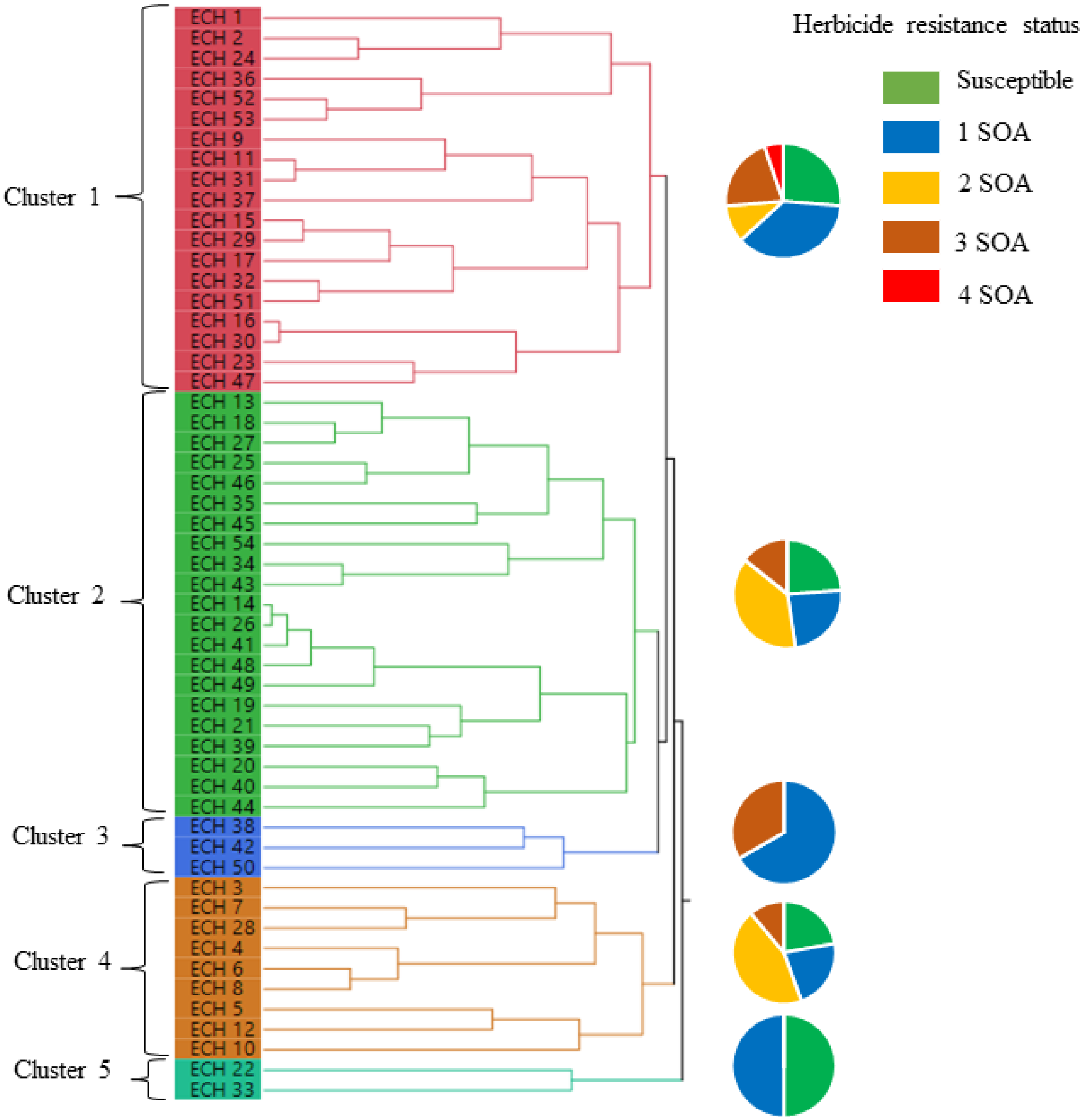

Figure 11: Hierarchical cluster analysis of 54 *Echinochloa* spp. populations with resistance profiling. Each color in pie- chart represents resistance to different number of sites of action (SOA)

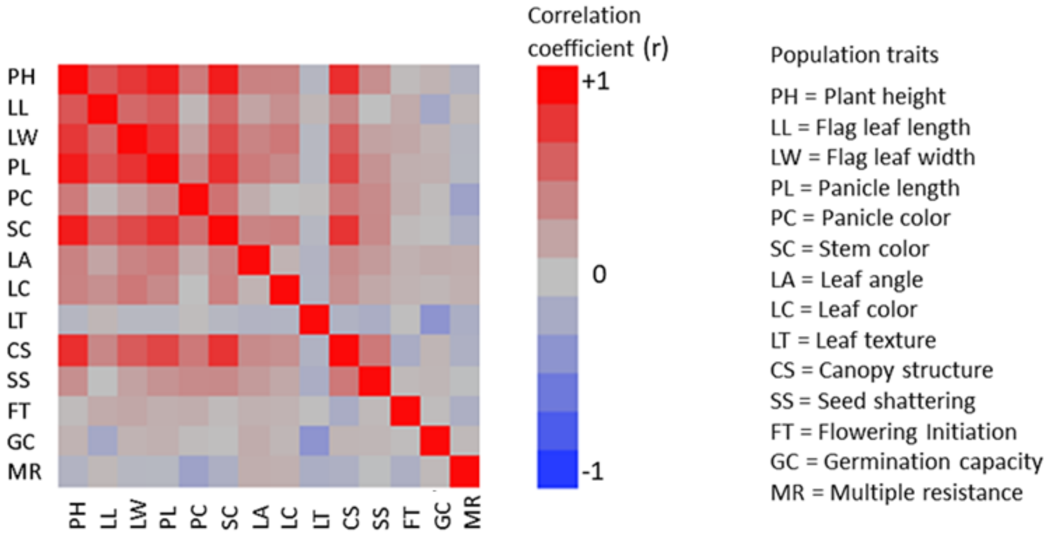

Figure 12: Correlation analysis for multiple resistance and 13 morpho-physiological characteristics measured in 54 *Echinochloa* spp. ecotypes surveyed across Texas.

CHAPTER V

SUMMARY AND CONCLUSION

The current studies provide insight into the evolutionary changes in morpho-physiological characteristics of *Echinochloa* spp. which is very important for evaluating their competitive ability and management in general. Traits like plant height, flag leaf length, seed shattering and seed germination contributed the most to the diversity of *Echinochloa* ecotypes. The results of this study have indicated that *E. colona* is the most dominant *Echinochloa* spp. in rice production in Texas. Most of the *Echinochloa* spp. populations have evolved resistance to one or more herbicide site of action and majority of the weedy rice populations were highly resistant to imazethapyr (ALS-inhibitor). The profiling of herbicide resistance in Texas rice weeds were documented in this study. The information on herbicide resistance of *Echinochloa* spp. and weedy rice may help in diversifying herbicide management programs to delay herbicide-resistance evolution. Information received through stakeholder questionnaire-survey on current weed management practices in Texas rice production, problematic weed issues, and concerns for herbicide resistances, would help in setting research and extension goals for future research needs.

APPENDIX

Supplementary table 1. Correlation analysis for multiple resistance and 13 morpho-physiological characteristics measured in 54 *Echinochloa* spp. populations surveyed across Texas

Population traits[a,b]	PH	LL	LW	PL	PC	SC	LA	LC	LT	CS	SS	FT	GC	MR
PH		0.65*	0.79*	0.92*	0.45*	0.91*	0.41*	0.40*	-0.09	0.83*	0.34*	0.03	0.10	-0.12
LL	0.65*		0.56*	0.64*	0.07	0.56*	0.2	0.33*	0.07	0.38*	0.00	0.15	-0.23	0.05
LW	0.79*	0.56*		0.81*	0.24	0.70*	0.40*	0.47*	-0.07	0.63*	0.21*	0.18	0.10	-0.08
PL	0.92*	0.64*	0.81*		0.38*	0.83*	0.45*	0.37*	-0.08	0.73*	0.31*	0.13	0.11	-0.08
PC	0.45*	0.07	0.24	0.38*		0.50*	0.13	-0.01	0.03	0.46*	0.36*	0.14	0.03	-0.28*
SC	0.91*	0.56*	0.71	0.83*	0.50*		0.40*	0.42*	-0.08	0.81*	0.37*	0.04	0.01	-0.16
LA	0.41*	0.20*	0.40	0.45*	0.16*	0.40*		0.09*	-0.12	0.36*	0.26	0.11	0.14	0.13
LC	0.40*	0.33*	0.47	0.37*	-0.01*	0.42*	0.09		-0.11	0.32*	0.17	0.09	0.07	0.11
LT	-0.09	0.07	-0.07	-0.08	0.03	-0.08	-0.12	-0.11		-0.14	-0.18	0.01	-0.40*	-0.17
CS	0.83*	0.39*	0.63	0.72*	0.46*	0.81*	0.36*	0.32	-0.14		0.46*	-0.18	0.08	-0.15
SS	0.33*	0.01	0.21	0.31*	0.36*	0.37*	0.26	0.17	-0.18	0.46*		0.05	0.07	-0.01
FT	0.03	0.15	0.18	0.16	0.13	0.041	0.11	0.09	0.01	-0.18	0.05		0.03	-0.16
GC	0.09	-0.23	0.09	0.11	0.03	0.01	0.14	0.07	-0.40*	0.08	0.07	0.03		0.05
MR	-0.12	0.05	-0.08	-0.08	-0.28*	-0.16	0.13	0.11	-0.17	-0.15	-0.01	-0.16	0.05	

[a] PH = Plant height, LL = Flag leaf length, LW = Flag leaf width, PL = Panicle length, PC = Panicle color, SC = Stem color, LA = Leaf angle, LC = Leaf color, LT = Leaf texture, CS = Canopy structure, SS = Seed shattering, FT = Flowering initiation time, GC = germination capacity, MR = Multiple resistance

[b] Significance of the effects is denoted by (*) at $P \leq 0.05$