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Evaluation and Characterization of Fitness Costs in Multiple Herbicide Resistant Echinochloa in Arkansas

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Evaluation and Characterization of Fitness Costs in Multiple Herbicide Resistant *Echinochloa* in Arkansas

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Dedication

This thesis is dedicated to my mother, Gail Penka, father, Lynn Penka, and my husband, Kevin Mills for all of their love, support, and encouragement.

Chapter 1: Introduction

Introduction

Ecologists have investigated the ability of plants to adapt to different environments or new environmental pressures, and they have credited survival in adverse conditions to genetic variability within a population (Grimes 1997). In a review by Muller-Sharer et al. (2004) about invasive plant evolution, resistance is defined as any mechanism plants use to reduce injury by herbivores, which eventually leads to coevolution of species. A prime example of how genetic variability within a plant population has caused coevolution can be observed with human and weed interactions. The earliest methods of weed control were manual weeding, and/or separating weed seed from crop seed after harvest. This caused selection for weed seeds or plants that were morphologically similar to the crops (Gould 1991). The introduction of plows and machines led to mechanical implements being used instead of manual labor, and this shifted weed populations to those with growing points below the ground or those with the ability to regenerate (Swanton et al. 1993; Shrestha et al. 2001). The introduction and use of herbicides in the 1950s allowed producers to control weeds in more time-efficient ways than had ever been done before, but by 1957 the first case of an herbicide-resistant weed was identified (Heap 2016).

The Weed Science Society of America (1998) recognizes resistance trait as being heritable, and plants that can reproduce after exposure to herbicide doses that usually kill the wild type. Herbicide resistance falls into two broad categories of resistance mechanisms; target site resistance (TSR) and non-target site resistance (NTSR). TSR pertains to the occurrence of a mutation or mutations in the herbicide target protein, which reduces or completely eliminates the affinity of the herbicide molecule to the target binding site, making the herbicide ineffective or less effective. TSR involves alteration of a single gene target, which generally endows high resistance levels. In some cases, the mutation results in only a slight change in the 3-D

configuration of the binding site, resulting in low-level resistance. NTSR involves mechanisms that do not directly involve a specific herbicide target. Examples of NTSR mechanisms are reduction in herbicide uptake in the plant due to thicker cuticle, reduced translocation, detoxification of the herbicide molecule, or sequestering the herbicide in the cell walls or in an organelle like the vacuole where it can no longer reach the active site (Jugulam and Godar 2013; Darmency et al. 2014). Some NTSR mechanisms such as alteration in herbicide entry and transport in the plant by different means (which may involve multiple genes) can endow only low-level resistance. However, the ability of some plants to detoxify herbicide molecules quickly have resulted in high levels of resistance, as is the case of propanil resistance in *Echinochloa* spp (Darmency et al. 2014). It has also been discovered that NTSR mechanisms enables plants to evolve resistance to multiple herbicides. One way this can happen is by the inadvertent repeated exposure of plants to sublethal doses of herbicides for various reasons (drift, large plant size, poor coverage, others), thus resulting in the accumulation of adaptation genes such as those for detoxification of oxidative compounds. In this situation, the plant can even develop resistance to low rates of chemicals that have never been applied to the field before (Yu and Powles 2014).

Since NTSR mechanisms include the amplification of genes, and sequestration, or detoxification of herbicides, researchers have been investigating if there are fitness costs associated with plants having these resistance mechanisms. Atrazine has 231 unique cases of herbicide resistance documented (Heap 2016). This herbicide has one of the highest numbers of resistance cases. In the majority of cases, resistance to atrazine was due to; a mutation in the *psb*A gene, which actually reduces the rate of electron transfer in Photosystem II. Consequently, the resistant plant incurs oxidative damage and expends more energy in renewing the D1 protein

in PSII, resulting in smaller plants with less seeds compared to the susceptible counterparts (Holt et al. 1993). Other examples of fitness cost associated with TSR were reported in some ACCase and ALS resistance species. It is difficult to quantify fitness cost associated with specific NTSR mechanisms, primarily because in many cases, the specific mechanism is not known, or multiple mechanisms occur in one plant. For example, reduced translocation of a herbicide in a resistant plant (a common NTSR mechanism) is the manifestation of many possible causes. Nonetheless, there are experimental methods that can be used to quantify the overall impact of NTSR (notwithstanding the specific mechanism) on plant fitness (Vila-Auib et al. 2005 and Menchari et al. 2008).

If certain resistance-conferring mutations cause reduced fitness, it is possible to adapt weed management methods to manage the weed population and potentially revert to a sensitive population. This idea of management would be applicable to weed species with seeds that do not persist in soil seedbank for extended periods, such as small-seeded annual grasses (Dawson et al. 1975). A good candidate for this approach would be small-seeded *Echinochloa* spp. (Williams et al. 1995) such as *E. colona or E. crus-galli*. The seed has little carbohydrate reserves, and the testa coat is not as thick and impermeable to water and gases as other troublesome weed species like *Amaranthus hybridus* (Gardarin et al. 2010).

Echinochloa crus-galli causes substantial losses in rice, cotton, and corn fields, not only in the United States, but also other countries as well (Bagavathiannan et al. 2011). In Arkansas in particular, *Echinochloa* infests almost all rice production acres and is the number one weed of rice in the United States (Norsworthy et al. 2013). The *Echinochloa* genus, collectively, has evolved resistance to six herbicide modes of action in 22 countries (Heap 2016). *Echinochloa* is troublesome for farmers in every one of these countries due to its ability to survive across diverse

environments (Juliana et al 2010). Mechanical or biological control methods in rice are costly, time-consuming and usually ineffective. Manual weeding is also ineffective due to the similarity of *Echinochloa* to rice at the seedling stage and the sheer large area of rice production (Jha et al. 2010). Therefore, herbicides have become the primary means of control for *Echinochloa* and other weeds.

The evolution of multiple resistance to herbicides is steadily increasing. It can be assumed, erroneously, that accumulating multiple mutations to acquire multiple resistance traits is physiologically benign. Despite various research activities dedicated to understanding the impact of resistance on weed fitness, there is no information on the impact of multiple resistance on plant function, productivity, or fecundity. This study will provide insight into fitness differences between multiple-resistant *Echinochloa* ecotypes and their susceptible counterparts. The general objectives include: 1) characterization of germination behavior between R and S *Echinochloa*; 2) characterization of vegetative and reproductive traits of R and S ecotypes; and 3) evaluation of competitive abilities of R and S ecotypes.

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Chapter 2: Literature Review

Echinochloa as a Weedy Issue

Rice (*Oryza sativa*) is an important crop in the south-central region of the United States. One of the weeds that causes the biggest financial losses for U.S. rice farmers is *Echinochloa* (Norsworthy et al. 2013). The negative impact of *Echinochloa* on the crop is not felt solely in U.S. rice fields. In over 60 countries and in a variety of crops, weedy *Echinochloa* species are problematic (Juliana et al 2010). *Echinochloa* is a C4 plant that is adaptable to a broad range of environments and is highly competitive with rice and other C3 crops (Bagavathiannan et al. 2012). It is more efficient in using water, has higher CO2 compensation point, and has a higher light saturation point. In drought or high light/temperature conditions, *Echinochloa* can regulate its temperature more effectively and keep producing assimilates from photosynthesis more efficiently than rice. All of these traits allow *Echinochloa* to survive in a wide range of environments such as flooded rice or upland crop fields.

In the 1990s the first cases of *Echinochloa* with resistance to propanil were discovered (Talbert and Burgos 2007). Since then, resistance to eight other modes of action has evolved among *Echinochloa* populations worldwide (Heap 2017). There has been confirmed resistance to the three commonly herbicide used modes of action in rice production, ALS (imazethapyr), ACCase (cyhalofop), and synthetic auxin (quinclorac) (Rouse et al. 2018). Resistance to herbicides (or other pesticides) is not a new phenomenon. *Echinochloa*, like other weedy species, has been adapting to crop management practices since the beginning of crop cultivation. The earliest methods of weed control involved manual weeding, and the *Echinochloa* ecotypes that looked most similar to rice was overlooked and left in the field. These plant types gradually became dominant, making the *Echinochloa* population in rice fields more and more similar to the rice cultivar with which it grew (Talbert and Burgos 2007).

Crop rotations have a significant impact on the size of *Echinochloa* seed bank (Bagavathiannan 2011). It was found that there was less *Echinochloa* seed in fields that had been in consistent cotton production and this was attributed to the intensive herbicide applications required to achieve clean culture. Conversely, fields that had been planted with soybeans most years had more *Echinochloa* seed in the seedbank. This is because farmers generally make less herbicide applications to soybean compared to cotton, and do not consider it necessary nor economical to control late-emerging weeds (including grasses). *Echinochloa* viability within the soil seedbank dropped significantly in a 4-year period (Egley and Chandler 1983). If the current resistant ecotypes of *Echinochloa* can be controlled and reduced in the soil seedbank, producers could see dramatic decline in resistant populations, or *Echinochloa* in general, within a few years.

Evolution of Resistance to Herbicides

Herbicide resistance problems plague 66 countries on six continents and impact crop production worldwide (Heap 2016). Several factors contribute to resistance evolution. First is high genetic variability, which favors the accumulation of beneficial mutations that eventually lead to adaptation to biotic and abiotic stresses, including adaptation to herbicide selection pressure (Tranel and Wright 2002). Second is herbicide misapplication due to biotic or abiotic factors. Third, is the use of the same herbicide or herbicide mode of action continuously across many growing seasons (Powles and Yu 2010). These principles were demonstrated by Yu and Powles (2014). In their research, herbicide-susceptible populations of *Lolium rigidum* (rigid ryegrass) were sprayed with low rates of diclofop. The surviving "susceptible" individuals were allowed to hybridize and reproduce. After three more iterations, this resulted in a diclofop-resistant population. The genetic variability within this ryegrass population allowed for

mutations that favored tolerant individuals to survive. Repeated exposure to sublethal doses of the same herbicide advances the selection for tolerant individuals. Ecologists and weed scientists alike have noted and studied the genetic variability within plant populations and how this has enabled plant survival (Grimes 1997; Powles and Yu 2010; Yu and Powles 2014). Some abiotic environmental factors that enable selection for resistance include cloudy, rainy, or windy days or dry conditions, which reduce herbicide efficacy. These result in suboptimal herbicide activity, which allows some individuals to survive sublethal amounts of active ingredient that reach the herbicide target. Mechanical and chemical factors both contribute to the selection for resistance to herbicides. These include aspects related to herbicide application such as improper calibration, nozzle type, spray volume, herbicide dose, antagonistic herbicide mixtures, and others. One extreme of these could result in a sublethal dosage received by any given plant resulting in unintended low-dose selection.

Resistance Mechanisms

Weedy species have evolved different resistance mechanisms that inhibit herbicides from reaching or binding to its target site. These resistance mechanisms are lumped into two broader classes, target site resistance (TSR) and non-target site resistance (NTSR) (Powles and Yu 2010). A TSR mechanism involves altering the target protein of the herbicide, causing it to be less sensitive or completely insensitive to the herbicide. A NTSR mechanism involves a single mechanism or a combination of mechanisms that decrease herbicide uptake and translocation or allows the plant to detoxify the herbicide before it reaches the target site. The NTSR mechanisms include; 1) decreased penetration into the plant, 2) decreased translocation 3) increase in sequestration of the herbicide, or 4) detoxification (Jugulam and Godar 2013; Powles and Yu 2010). The diclofop-resistant rigid ryegrass developed in Australia (Yu and Powles 2014),

through recurrent low-dose selection, was able to metabolize diclofop and other dissimilar herbicides, showing broad resistance by NTSR mechanism.

The inheritance pattern of herbicide resistance and the mating behavior of resistant species impact the spread of resistance issues. It has been discovered that TSR and NTSR are inherited as either dominant or semi dominant genes dependent on species (Powles and Yu 2010). NTSR involves mutations at several loci and multiple genes or gene elements, and therefore has complex inheritance patterns that could not be resolved to a specific gene. TSR usually involves altering only the gene associated with the herbicide target and therefore is easier to study (Jugulam and Godar 2013). Plants harboring TSR mechanism can typically withstand a higher rate of a particular herbicide than those expressing NTSR mechanisms (Darmency et al. 2014). However, this generalization may be flawed because no studies have compared the resistance level of one species to one herbicide by TSR and NTSR mechanisms.

The following sections focus on resistance evolution to four herbicide modes of action used in managing *Echinochloa spp*. in US rice production. These are ACCase-inhibitors (acetyl-CoA carboxylase), ALS-inhibitors (acetolactate synthase), synthetic auxins, and photosystem II inhibitors.

ACCase Resistance

ACCase-inhibitor herbicides inhibit the first step of fatty acid biosynthesis, effecting slow plant death by depriving the plant of ingredients to synthesize lipids for structural, growth, storage, transport, or defense purposes (Kukorelli et al. 2013). It is effective only on grasses due to the presence of a sensitive ACCase isozyme in the plastid of poaceae. Three classes of herbicides target ACCase including aryloxyphenoxy propionate (fops), cyclohexanedione (dims), and phenylpyrazoline (dens) (WSSA 2008; Kukorelli et al. 2013). The newest class in this

chemistry, phenylpyrazoline, was discovered in 2006 and can be used in certain cereal crops. The ACCase-inhibitor chemistry started being widely used in the 1980s (Kukorelli et al. 2013). The first cases of ACCase resistance were identified in 1982 in the United Kingdom with *Alopecurus myosuroides* (blackgrass) and in the same year in Australia with rigid ryegrass (Heap 2016). Since then cases of ACCase resistance has climbed steadily. The first case of ACCase resistance in the United States was documented in 1987 with *Lolium multiflorum* (Italian ryegrass). There are currently 47 species worldwide that have documented ACCase resistance; five of these belong to the genus *Echinochloa*; three species, junglerice (*Echinochloa colona*), barnyardgrass *(Echincochloa crus-galli)*, and late watergrass (*Echinochloa phyllopogon*) (Heap 2018).

The majority of resistance to ACCase inhibitors is due to a mutation or mutations in the target site (TSR mechanism). Blackgrass is one of the most notorious species with resistance to ACCase inhibitors; the mechanism is primarily TSR. There are currently eleven amino acid substitutions that are associated with ACCase resistance (Kukorelli et al. 2013). Menchari et al. (2006) studied three of these mutations, Gly_{2078}, Asn_{2041}, and $Leu_{1781.}$ The objective was to determine how frequently these three mutations occurred in blackgrass populations across Europe. It was found that Gly_{2078} occurred less frequently while Leu_{1781} occurred most often among ACCase-resistant blackgrass populations. The Gly_{2078} mutation was found to be resistant to all fops and dims in the ACCase herbicide group. The Asn_{2041} mutation was noted to endow resistance to all ‘fops’ but not to ‘dims’. Meanwhile the Leu_{1781} mutations was observed to endow resistance only to some ‘fops’ and ‘dims’ (Menchari et al. 2008).

This seems to agree with the findings that the Gly_{2078} mutation causes some fitness penalty while Leu_{1781} mutation is inconsequential to plant fitness. A plant that is less fit will not

be able to compete with normal plants and will not flourish as much. In addition, the low frequency of Gly_{2078} genotype in the population could also be a consequence of it being a recessive trait. This means that a resistant individual will exist only if the plant is homozygous resistant. Another study performed by Darmency et al. in 2014 did not focus only on the single locus mutation but also considered the background genetic variation of populations harboring one of the three mutations (Leu_{1781}, Asn_{2041}, and Gly_{2078}) and susceptible populations. They used 13 accessions to form three groups of genotypes plus the susceptible group. In this study they observed that the Gly_{2078} mutation group had slightly higher vegetative biomass and seed production than the other genotype groups (Darmency et al. 2014). The disparity between studies could be due in part to different sample sizes and different places of origin of accessions. In both studies only two accessions with the Gly_{2078} mutation were used while the other two mutation groups, Leu_{1781} and Asn_{2041}, and the susceptible group had three to five accessions. The background genetic variance could not be accounted for in fitness studies if the resistant and susceptible plants are derived from different locations. Recombinant inbred lines or near isogenic lines of R and S plants are ideal for studying fitness costs of target site mutation(s). Creating these takes time and additional resources. One alternative is using a resistant and susceptible ecotype derived from the same population to minimize the confounding effect of genetic variance on plant fitness. The identified NTSR mechanisms associated with ACCase resistance are; herbicide detoxification via cytochrome P450 monooxygenases, and increased activity of ACCase which, for example, was documented in R *Sorghum halepense* (Kukorelli et al. 2013).

However, in the case of this proposed study, not knowing the exact resistance mechanism can prevent the total understanding of fitness consequences. In both the studies performed by Darmency et al. (2014) and Menchari et al. (2008) showed that Gly_{2078} mutation resulted in

reduced vegetative biomass and seed production in a year with unfavorable growing conditions. This indicates that stressful environments could render some resistant populations less fit and less competitive, thereby favoring the dominance of sensitive plants in the next generation.

ALS Resistance

The inhibition of the ALS biosynthesis pathway leads to deficiency in amino acids, leucine, isoleucine, and valine (Saari et al. 1990). Without these essential amino acids several plant processes will shut down. This group of herbicides (Group 2) has been widely used since the decade of commercialization of the imidazolinone and sulfonylurea family of ALS inhibitors in the 1980s. These herbicides became popular very quickly in major agronomic crops because of their low-use rates, high level of efficacy, mostly broad spectrum of activity, and generally moderate to long residual activity. A few ALS inhibitors have been used, in small volumes, in rice production (Shivrain et al. 2007; 2008; Bagavathiannan et al. 2014). Imidazolinones (i.e., imazethapyr and imazamox in the US) were introduced in the rice production system with Clearfield rice technology in 2002, which increased the use of ALS herbicides drastically in rice. These 'new' and widely used ALS herbicides include Newpath, Clearpath, and Beyond. Hardke (2015) reported that 47% of Arkansas rice acres were planted with Clearfield rice varieties.

Resistance to ALS herbicides is the most widespread resistance problem worldwide (Heap 2016). The first case of ALS resistance was documented in 1982 in rigid ryegrass in Australia. In 1987, three unique cases of ALS resistance were identified within the United States, kochia (*Kochia scoparia*), prickly lettuce (Lactuca serriola), and Russian thistle (*Salsola tragus*). The current number of species resistant to ALS herbicides is 158. There are currently five ALS-resistant *Echinochloa* species; three of these are in the United States, junglerice, barnyardgrass, and late watergrass. Barnyardgrass and junglerice infest rice fields in the southern in the United

States. Since the introduction of the Clearfield technology, ALS-resistant barnyardgrass and junglerice has been increasing in Arkansas.

The ALS group of herbicides are associated primarily with TSR (Panozzo et al. 2013; Sales et al. 2008). To date, only three ALS-resistant weed species have been confirmed to have NTSR mechanisms; smooth pigweed (*Amaranthus hybridus*), (wild mustard) *Sinapis arvensis,* and common poppy (*Papaver rhoeas*) (Scarabel et al. 2015). The heritability of NTSR mechanisms is not understood, and there is a lack of understanding of the process by which each mechanism is inherited. It is simply demonstrated that the resistance is not due to a single point mutation in the ALS alleles. There are around 670 amino acids that comprise the ALS protein (Oard et al. 2011). There are over 22 mutations in eight of these 670 amino acids that endow ALS resistance (Heap 2016). The amino acid with the most (7) nucleotide substitutions across the most number of weed species is Pro_{197}. Some weed species exhibit multiple mutations in the site of action. The most mutations, 5, occur in grass species like blackgrass (Heap 2016).

Photosystem II Inhibitors

Propanil belongs to the amide class of herbicides (Group 7), which inhibit electron transport in photosystem II. Propanil was synthesized in 1957 and commercialized in 1962 for rice cultivation (Hoagland et al. 2004). The first unique case of propanil resistance was documented in 1988 in Colombia in junglerice. Since then, 28 unique cases of resistance to propanil have been identified; 23 of these cases involve species from the *Echinochloa* genus (Heap 2016). Due to increasing farm sizes and increasing labor costs, rice producers in the southern US turned to propanil for broad spectrum postemergence weed control in rice (Bagavathiannan et al. 2011). Among susceptible barnyardgrass populations, control with propanil is excellent (>95%), but drastically reduced in mid-to-late season applications (Scott et

al. 2016). This poor control in the later season applications must have contributed to *Echinochloa* resistance evolution; the late applications allowed for low-dose exposure to propanil, leaving survivors that produced seed.

It has been demonstrated that the resistance mechanism to propanil in barnyardgrass is via NTSR, involving detoxification of propanil by an aryl acylamidase enzyme (Hoagland et al. 2004). This degrades propanil to 3,4-dichloroaniline plus propionic acid (Frear and Still 1968). The insecticide carbaryl is known to inhibit the aryl acylamidase enzyme. The inhibition of this enzyme makes a resistant plant sensitive to propanil. Applying propanil with carbaryl to rice results in crop injury (Hoagland et al. 2004).

Synthetic Auxins

Quinclorac is a systemic synthetic auxin herbicide (Group 4) used to control some monocot and dicot weeds in rice production. The action of this herbicide mimics the overdose of auxin by affecting the phytohormonal system and causes shoot stunting and twisting (Grossmann and Kwiatkowski 2000). The other synthetic auxin herbicides in Group 4 do not typically affect grasses, but quinclorac is highly effective on barnyardgrass and junglerice (Grossmann and Kwiatkowski 1995). It was discovered that sensitive species, e.g. barnyardgrass, exposed to quinclorac accumulates cyanide in the shoot tissues, and ultimately also in the roots (Grossmann and Kwiatkowski 1995; Lamoureux and Rusness 1995; Koo et al. 1997).

Continuous use of propanil led to resistant ecotypes of *Echinochloa*, Arkansas rice producers started using quinclorac. By the late 1990s some barnyardgrass populations had evolved resistance to both propanil and quinclorac (Talbert and Burgos 2007). There are currently eleven unique cases of resistance to quinclorac worldwide; the *Echinochloa* genus constitutes nine of these, and two cases involve barnyardgrass in the United States (Heap 2016).

Barnyardgrass populations that are resistant to quinclorac were believed to have a TSR mechanism (Grossmann and Kwiatkowski 1995; 2000).

The evolution of quinclorac-resistant *Echinochloa* led to yet another loss of a mode of action for rice producers. Research on utilizing tank mixes of herbicides for control options of quinclorac-resistant *Echinochloa* have been conducted, but none were effective on resistant populations.

Fitness Costs

Studies have been conducted to understand how herbicide resistance can impact plant fitness. Fitness is determined by the ability of a plant to successfully germinate and reproduce in certain environmental conditions (Gould 1991). Understanding fitness helps with predicting population dynamics and how to better manage resistant populations. The genetics endowing resistance play a big role in understanding fitness costs. There have been studies conducted where the genetic background contributing to resistance was not identified and fitness costs were not observed (Menchari et al. 2008).

Fitness costs have been associated with resistance to triazine herbicides. In a study performed on the combined effects of ALS- and triazine resistance in mat pigweed (*Amaranthus blitoides*), all the resistant ecotypes exhibited some reduced fitness in the replacement studies. This growth penalty associated with the resistance was not significant though, and the researcher concluded that the minor fitness penalties were due to the triazine resistance and not the ALS resistance (Sibony and Rubin 2002). The ALS herbicides are commonly associated with TSR mechanisms (Panozzo et al. 2013). Studies conducted on understanding how these TSR and NTSR mechanisms affect fitness have shown that TSR generally have no fitness effects while the consequences of NTSR are not fully understood (Vila-Auib et. al 2005; Menchari et al. 2008;

Bagavathiannan et al. 2011; Rosenhauer et al. 2014). Another mode of action that is commonly associated with TSR is the ACCase herbicide target. Menchari et al. (2008) conducted experiments with black-grass populations with three of the seven most noted mutations, Leu_{1781}, Asn_{2041}, and Gly_{2078}. After collection of eight accessions in eastern France, they identified the resistance-conferring ACCase mutations and created 10 populations with a single mutation and with combinations of the three alleles. They then characterized the plants based on height of the tallest tiller, biomass after removal of seed, and the amount of seeds produced. The length of one panicle and number of spikelets were also measured and analyzed the correlation of these traits with seed production. They estimated seed production based on the sum of the lengths of all the inflorescences. Mutations at Leu_{1781} and Asn_{2041} did not cause reduced fitness. Mutation at Gly_{2078} resulted in fitness penalty with respect to plant height, vegetative biomass, and overall seed production. The fitness costs associated with this allele were minor; therefore, it was assumed to be a recessive trait. This was the first study to examine how a mutation at a particular locus influences fitness. The fitness cost of resistance to propanil or quinclorac is not well studied. Bagavathiannan et al. (2011) reported that there were no fitness costs associated with resistance to propanil or quinclorac in the *Echinochloa* population tested. The effect of multiple resistance on plant fitness was not investigated. Although no fitness cost was observed with quinclorac-resistant barnyardgrass, quinclorac-resistant wild mustard were less fit than the wild type (Jugulam and Godar 2013).

Environmental and habitat conditions will accentuate variability among individuals in the absence of herbicide selection, and ultimately lead to different levels of fitness. Some studies have reported fitness costs due to a particular resistance mechanism, but the genetic background was not accounted for (Cousens et al. 1997). Cousens, Gill, and Speijers (1997) further

commented on the importance of choosing resistant and sensitive of similar genetic background, and also stressed the importance of choosing the correct number of populations to study. Cousen et al. (1997) stated that to observe 10% effect on fitness at an 80% chance of detection and 5% level of significance, 18 populations of each ecotype are needed. This is a daunting number of populations to not only collect but also to characterize in terms of resistance level and fitness traits. The review also stated that three ecotypes of each would be needed to detect a 30% fitness effect. One disadvantage of selecting few representative ecotypes would be the loss of power to detect a 10-20% effect on fitness.

Another issue that arises in most resistance studies and is most frequently critiqued is the lack of consideration for compensatory evolution. It usually takes multiple generations of resistance before a producer notices resistance in a field. Adaptive selection could have already occurred by this time, so it is impossible to tell if negative effects of mutations, fitness costs, have already been selected against. In this current proposed study, it is impossible to determine the extent to which compensatory evolution has occurred among the accessions used; if this study shows no fitness costs, we could not rule out the possibility that some background compensatory evolution has occurred before the accessions were collected.

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Chapter 3: Characterization of herbicide-resistant *Echinochloa colona* in Arkansas

Abstract

Echinochloa spp. are major weed problems for rice (*Oryza sativa* L.) on a global scale. In the southern rice belt of the U.S.A., *Echinochloa colona* (L.) Link (junglerice) and *E. crus-galli* (L.) P. Beauv (barnyardgrass) are the most troublesome weeds. These species are morphologically diverse, and its high ploidy level augurs high genetic diversity, which favors high adaptability to stress, including herbicide selection pressure. The objectives of these studies were to 1) isolate resistant (R) and sensitive (S) biotypes from eight accessions of *E. colona* with different resistance profiles; 2) determine the resistance levels of selected R and S biotypes of each accession; and 3) characterize the vegetative and reproductive traits of these plants.

Populations with different levels of resistance to propanil and quinclorac were characterized. Quinclorac was the least effective on most accessions, with all plants in Eco-45 and Eco-76 surviving at 36,164 g ai ha^{-1} (64x rate). The LD_{50} values ranged from 565-24,408 g ai ha^{-1} (1-32x rate). Propanil was slightly more effective than quinclorac with LD_{50} values ranging from 3,991-80,730 g ai ha^{-1} (approximately 1-16x rates). All accessions were susceptible to cyhalofop. Since segregation of biotypes into R and S biotypes was not successful, biotypes with high resistance levels (R1) and biotypes that had lesser resistance (R2) were used. The R1 and R2 biotypes did not differ in the number of seeds per panicle, except for Eco-35. The Eco-35R1 biotype produced 23% more seed than its R2 counterpart. The number of days to seed shattering did not differ between biotypes, except for Eco-208 where seeds of R1 plants shattered 2 wk later than those of R2 plants. Therefore, except for delayed maturation, the R1 biotypes did not exhibit fitness penalty in terms of fecundity under optimum growing conditions, with the exception of Eco-35R1.

Introduction

Echinochloa species are major weed problems in a variety of crops throughout the world, but are the most problematic grass weeds in rice (Chauhan et al 2017; Juliana et al. 2010; Norsworthy et al. 2013). *Echinochloa* spp. cause yield losses ranging from 68% (barnyardrass) to 85% (junglerice) and can cause the biggest financial loss for rice producers (Chauhan 2017; Norsworthy et al. 2013; Smith 1988). Barnyardgrass and junglerice can grow in both flooded and upland environments hence it is a problem in rice and upland crops (Bagavathiannan et al. 2011; Chauhan et al. 2017). The expansion of US rice production in the midsouth and into the west coast in California was facilitated by the availability of effective rice herbicides for annual grass control. The first major herbicide used to control the junglerice/barnyardgrass complex was propanil.

Propanil became the backbone of chemical weed control in rice because of its broad-spectrum activity, wide window of application, and flexibility in tank-mix partners (Lovelace et al. 2003). Since its commercialization in 1959, propanil has been sprayed on practically every rice field in the U.S. In the late 1980s, Arkansas rice farmers started notifying Extension Agents about occasional failures of propanil in some fields (Talbert and Burgos 2007). In the 1990s, barnyardgrass with multiple resistance to propanil and quinclorac was confirmed within the state of Arkansas (Lovelace et al. 2003, Talbert and Burgos 2007). Since then, resistance to multiple modes of action (MOAs) has been reported to imazethapyr, propanil, and quinclorac as well as single resistance to clomazone in the junglerice/barnyardgrass complex in Arkansas and the US mid-south (Heap 2017).

Resistance to herbicides occurs by two broad categories of mechanisms: target site resistance (TSR) and non-target site resistance (NTSR) (Powles and Yu 2010). Non-target site

resistance involves either detoxification of herbicides, reduced absorption or translocation, increased protection from herbicide damage, or amplification of the herbicide target gene (De'lye et al. 2013). These mechanisms involve multiple genes and are difficult to attain; therefore, NTSR traits may be associated with fitness costs (Darmency et al. 2014; Jugulam and Godar 2013). Target site resistance involves alteration of the herbicide target gene, some of which may also carry some fitness penalty because of reduced efficiency of the mutant target enzyme (Bagavathiannan et al. 2011; Menchari et al. 2008; Vila-Auib et al. 2005; Vila-Auib et al. 2009)

There are at least four species of *Echinochloa* in the state of Arkansas: barnyardgrass, junglerice, rough barnyardgrass (*Echinochloa muricata*) (L.) P. Beauv, and coast cockspurgrass (*Echinochloa walteri*) (L.) P. Beauv (Tahir et al. 2016). Of the four species found in Arkansas, Tahir et al. (2016) reported that approximately 77% was junglerice; less than 10% was barnyardgrass. Junglerice and barnyardgass are commonly confused for one another and both have confirmed cases of single- and multiple-resistance to herbicides (Lovelace et al. 2003). This research focused on: 1) isolating resistant (R) and sensitive (S) biotypes from eight accessions of junglerice with different resistance profiles; 2) evaluating the resistance levels of each accession; and 3) characterizing possible differences between R and S biotypes in morphological traits and reproductive capacity.

Materials and Methods

Seed Source and Characterization. Seeds of junglerice and barnyardgrass were collected from producer fields at the end of the rice growing season between 2010 and 2015. The rice fields were selected based on reports from consultants and University Extension agents having populations that survived one or more herbicide applications. Panicles were collected

separately by site and plant type in each field. The sample sizes ranged from a few plants in a small patch to at least 200 g of seed from larger patches of one plant type. Seeds of these field-collected samples were stored at room temperature in paper bags until they were cleaned and tested for resistance. Postemergence herbicides used in both rice and soybeans at commercial application rates were used (Table 3.1). Ten seeds were planted in 10-cm by 10-cm pots filled with LC1 potting mix (Sun Gro Horticulture, Seba Beach, AB, Canada). Seedlings were sprayed at two-leaf stage with each herbicide mixed with the recommended adjuvant (0.25% non-ionic surfactant or 1% crop oil concentrate). Herbicides were applied in a spray chamber with a motorized boom fitted with two 8002 flat-fan nozzle tips (TeeJet Technologies, P.O. box 7900 Wheaton Il 60187) in a carrier volume of 187 L ha^{-1} at a speed of 1.6 kph and height of 75 cm above the plants. At three weeks after treatment (WAT), seedlings were evaluated for injury relative to the non-treated controls and the number of survivors counted (data not shown). Herbicide resistance profiles were determined for each accession based on these assays and eight accessions were selected for further studies based on the resistance profiles (Figure 3.1). These accessions were identified as junglerice based on their morphological traits (Tahir et al. 2016).

Isolation of susceptible plants from each accession. Ten seeds of each accession were planted into 10- x 10-cm pots, 10 pots per accession for each herbicide. Seedlings were thinned to one per pot. Each accession was sprayed with a low dose of each herbicide of interest to select the most susceptible plants in each accession using low doses of each herbicide applied at 3-4 leaf stage. At 3-4 leaf stage, the seedlings were sprayed with a 1x rate of propanil or quinclorac or ¼ x rate of cyhalofop, imazethapyr, or glufosinate based on results of previous bioassays of field-collected samples (Table 3.1). The low rates of some herbicides allowed selection of sensitive plants, which sustained injury, but survived and produced seed. Injury (stunting,

chlorosis, necrosis, reduced tillering) was evaluated 2 WAT, relative to the control plants. Individuals showing more than 60% injury were classified as sensitive (S biotype) while those with less than 30% injury were classified as resistant (R biotype). The selected S and R biotypes were then transplanted into 18- x 10-cm pots filled with the same volume of field soil (Captina Silt loam-fine-silty, siliceous, mesic Typic Fragiudults) from the Arkansas Agricultural Research and Extension Center Fayetteville, Arkansas. The R and S biotypes were grown for seed production in a greenhouse maintained at 32-37°C with 10-h light. Seeds were utilized in succeeding experiments. The offspring were subjected to dose response assays to determine the level of resistance.

Seeds harvested from plants identified as R or S from the original parent population via low-dose screenings were planted and three plants per biotype of each accession were characterized without herbicide treatment. The data collected were tiller height (measured from the soil surface to the tip of the flag leaf of primary tiller) and number of days to initiation of seed shattering. The latter was determined by recording the date when the tip of seeds on the three oldest panicles turned brown. The panicles were tapped with a constant force into a cup and the shattered seeds were weighed and counted.

Evaluation of resistance level of *E. colona* with different resistance profiles. Dose response bioassays were conducted in the greenhouse (32-37°C, 12-h days) at the Altheimer Laboratory, University of Arkansas, Fayetteville. Ten seeds from identified R and S plants were planted in 10- x 10-cm pots filled with LC1 commercial potting mix (Sun Gro Horticulture, Seba Beach, AB, Canada), 5 cm from the top. After emergence the seedlings were thinned to two, uniform-size plants per pot. Imazethapyr, quinclorac, and propanil were applied to 2-leaf plants at ten doses with their respective recommended adjuvants using the same spray chamber and

settings as in the previous section (Table 3.1). Treatments were replicated three times and the experiment was repeated temporally. Injury was assessed visually at 3 WAT and the number of survivors were counted.

Statistical Analysis

Resistance level evaluation. Regression curve fitting was done using Jmp PRO 13 (Statistical Analysis Systems Institute, SAS Circle P.O. Box 8000 Cary NC 25712-8000). Injury (%) and frequency of survivors (%) were fitted with four- and three-parameter sigmoid logistic models and low order polynomials. Some highly-resistant accessions had minimal response to the highest herbicide dose, used which in some cases did not require logistic response.

The general form of the regression equations are given by

(1) y= c+(d-c) / [(1+EXP(-a(Rate-b))]

(2) y= c / [(1+EXP(-a(Rate-b))]

where a is the exponential rate coefficient, b is the inflection point (or where the slope changes), c is maximum injury or percent of survivors (upper asymptote), and d is the lower asymptote (absent in the three-parameter).

(3) y=a*EXP(b*rate)

(4) y= a+b*EXP(c*rate)

(5) y= a+b*rate+c*rate^2

Where a is asymptote b is scale and c is growth rate.

Results and Discussion

Response of biotypes to dose response assays. After the initial segregation from field populations that showed differential response to herbicides in table 3.1, the dose response assays showed that each pair of biotypes per accession (except Eco-26) were both resistant (Table 3.2).

Since there were observable differences in injury between biotypes in some accessions, all biotypes were kept separate and were classified as highly resistant (R1) or resistant (R2) (Table 3.3). Eco-26 was not resistant to cyhalofop so the biotypes in this accession were classified as sensitive (S1) and highly sensitive (S2).

Response to cyhalofop. Bioassays of field-collected accessions in the greenhouse showed elevated tolerance to cyhalofop in Eco-26, Eco-45, Eco-208, and Eco-245 (Table 3.2-3.3). Survival at the 0.5x rate (297 g ai ha^{-1}) of cyhalofop was up to 54% and survival at the 2x rate (1189 g ai ha^{-1}) was up to 16% (Table 3.3). Of these accessions, Eco-45 was most resistant to cyhalofop. The LD_{50} for this population was 297 g ai ha^{-1}, which was approximately the 0.5x rate, and higher than those of the other accessions tested. In the field, some survivors are expected from the 1X rate of cyhalofop. The efficacy of cyhalofop on junglerice or barnyardgrass in rice production fields is generally around 80% (Scott et al. 2017). This means that, at any given location, there would be survivors that need to be controlled with supplemental herbicides. Cyhalofop, at 314 g ha^{-1}, can control barnyardgrass 90-100% in Arkansas (Jha et al. 2010). The bioassays indicate that cyhalofop is still effective on junglerice; however, suboptimal conditions and inadvertent exposure to sublethal doses of cyhalofop in the field can result in a large number of escapes in some populations. Scouting the fields after herbicide application and implementing follow-up control measures are necessary to avert weed control failures and selection for resistance. The first case of resistance to cyhalofop in the *Echinochloa* complex (barnyardgrass) within the United States was reported in 2000 in California (Heap 2018). Resistance to ACCase inhibitors among *Echinochloa* species is increasing gradually in the southern U.S. (Rouse et al. 2018). None of the ACCase-resistance cases were attributed to junglerice mainly because distinguishing between barnyardgrass and junglerice is difficult (Tahir

and Burgos 2016). It is possible that some reported cases of resistance were erroneously attributed to banyardgrass. In 2011, multiple resistance to fenoxaprop (another ACCase herbicide) and three other modes of action was confirmed in Mississippi in barnyardgrass (Heap 2018).

Response to quinclorac. Quinclorac applied at different rice growth stages controls barnyardgrass 80-90% in Arkansas when no resistant ecotypes are present (Scott et al. 2017). In this current research, quinclorac was the least effective of the four herbicides tested (including imazethapyr, propanil, and cyhalofop). Resistance to quinclorac is the second most common resistance problem among junglerice and barnyardgrass populations in Arkansas (Rouse et al. 2018). A few of the junglerice accessions in this study had very high levels of resistance to quinclorac. Injury at the 2x rate of quinclorac (1130 g ai ha^{-1}) ranged from 3-97% with 25-100% survivors across accessions (Table 3.3). Six of the eight field-collected accessions used in this research were resistant to quinclorac. Some accessions, including Eco-45, were not controlled with the highest rate tested (18,082 g ai ha^{-1} or 32x rate). This, and Eco-76 were not controlled even 50% with any rate tested (Table 3.2-3.3). High resistance to quinclorac is due to the intense selection pressure imposed on junglerice populations as this herbicide became the primary grass herbicide for rice after the outbreak of resistance to propanil (Talbert and Burgos 2007). There were no true quinclorac-sensitive plants in any of the tested quinclorac-resistant accessions, although R1 and R2 plants still differed in response to the herbicide (Figure 3.2a and 3.2b). In Eco-35, for example, the R2 plants had a LD_{50} of 791 g ai ha^{-1} (Table 3.2.) This is equivalent to 1.4x of the quinclorac field rate. Thus, the full rate cannot kill 50% of Eco-35R2 plants. The LD_{50} of Eco-35R1 was 30x higher than that of Eco-35R2 (Table 3.2, Figure 3.3). Quinclorac resistance in barnyardgrass has been documented since 1998 in Louisiana and dual resistance to

propanil and quinclorac in Arkansas was first documented in 1999 (Lovelace et al. 2003; Bagavathiannan et al. 2014, Heap 2017).

Response to propanil. Four out of eight accessions tested were resistant to propanil. In production fields, propanil is expected to provide 90% control of susceptible populations of barnyardgrass. This means that occasionally, there will be survivors due to variable environmental conditions at the time of application and variable plant sizes. The first unique case of resistance to propanil within the junglerice/barnyardgrass complex of Arkansas was documented in 1990 (Talbert and Burgos 2007). In the following years, it was observed that only 40% control of junglerice/barnyardgrass complex can be expected when propanil is applied without a tankmix partner (Scott et al. 2017). Rouse et al. (2018) reported that resistance to propanil was the most common resistance problem within the junglerice/barnyardgrass complex of Arkansas. In the current research, Eco-45 was the most resistant to propanil among the accessions tested, with Eco-45R1 showing a LD_{50} of 80,730 g ai ha^{-1}. This is equivalent to 18x of the propanil field use rate. As observed with quinclorac, Eco-45 no longer had individuals in the population susceptible to propanil. The LD_{50} for less-resistant plants in Eco-45R2 was 17x the field use rate of propanil. A 2x rate did not elicit any response from this population. The LD_{50} values of other accessions ranged from 3,991-56,062 g ai ha^{-1} (approximately 1-12x rate).

Response to glufosinate. The only accession with some tolerance to glufosinate was Eco-45. When treated with 0.5x rate glufosinate, Eco-45R1 showed only 14% injury among surviving plants while 96% of individuals were killed. Eco-45R2 had a 30% injury at this rate and only 22% of individuals were killed (Table 3.3). The LD_{50} values were 162 g ai ha^{-1} for Eco-45-R1 (more than 0.25x rate) and 134 g ai ha^{-1} (less than 0.25x rate) for Eco-45R2. We included glufosinate in this research because glufosinate-resistant soybeans are being planted in more

areas now to help manage herbicide-resistant weeds. Soybean is commonly planted in rotation with rice (Burgos et al. 2008). Therefore, junglerice populations in rice are exposed to glufosinate in the soybean crop when present at the time of application. However, since Liberty Link soybean has not been adopted widely for a long period in Arkansas, the selection pressure from glufosinate is relatively low compared to other herbicides. Differential tolerance to glufosinate (Rouse et al. 2016) is expected among the junglerice/barnyardgrass complex. A single application of glufosinate can provide 70% barnyardgrass control; it requires two applications to attain 90% grass control in soybean (Scott et al. 2017). Split applications may occasionally result in suboptimal herbicide use when the second application cannot be applied on time, or not at all, due to mitigating circumstances (weather conditions, equipment availability). When the grass gets too large, the second application will be ineffective, leading to low dose exposure and selection for more tolerant plants (Yu and Powles 2014).

Response to imazethapyr. Two of the eight accessions (Eco-101 and Eco-225) were resistant to imazethapyr and were not controlled across all the rates tested. At the 2x rate, Eco-101R1 and Eco-101R2 incurred 30% and 55% injury, respectively. Eco-225R1 and Eco-225R2 had 19% and 45% injury, respectively. Imazethapyr resistance in barnyardgrass has been reported since 2011 in Arkansas (Heap 2018). Susceptible populations in production fields can be controlled 90% with imazethapyr (Scott et al. 2017), but resistance to this herbicide is increasing in Arkansas (Rouse et al. 2018). Junglerice resistance to imazethapyr in rice is attributed to the widespread adoption of the Clearfield® rice technology. In 2017 around 41% of the rice planted within the state was Clearfield® rice (Hardke 2017).

Morphological characterization of accessions. The tallest accession was Eco-76 (118-125 cm), which was resistant to quinclorac only. The R1 biotype of Eco-76 was taller than the

R2 biotype, but this was not observed in any other accession (Table 3.4). In general, the number of seeds panicle^{-1} did not differ between biotypes of most accessions, except with Eco-35, which has multiple resistance to propanil and quinclorac. Eco-35R1 had 306 seeds panicle^{-1} and Eco-35R2 had 251 seeds panicle^{-1}. Tahir et al. (2016) found that in Arkansas, seed production of junglerice populations is highly variable, ranging from a few hundreds to over 1000 seeds per panicle. If populations with the same resistance profile and fecundity as Eco-35R1 are introduced into a field, the highly resistant biotype can increase in population size rapidly due to its higher seed production. Dual resistance to propanil and quinclorac has existed in southern USA rice fields since the 1990s (Talbert and Burgos 2007). The potential physiological consequences of having these resistance traits had not been studied in junglerice (or barnyardgrass). A competition study involving a barnyardgrass population with dual resistance to propanil and clomazone did not find differences in fitness between the susceptible standard population and the R ecotypes of different populations (Bagavathiannan et al. 2011). In this current study, a difference in seed size was observed only between Eco-45R1 and Eco-45R2; the less resistant biotype produced larger seeds than the highly resistant biotype based on 1000-seed weights (Table 3.4). Larger seeds have more energy reserves and, generally, are expected to last longer in the soil (Dawson and Bruns 1975). However, the difference in seed size between Eco-45R1 and Eco-45R2 is likely not large enough to influence seed longevity. It has been noted that ecotypes from the same region can have differences occurring between ecotypes due to the evolution of the ecotypes in the fields rather than the resistance mechanism to herbicide (Darmency et al. 2014).

Seeds of Eco-208R2 shattered earlier than Eco-208R1. This accession has resistance to quinclorac. Difference in maturation between biotypes was not observed in the other accessions.

The effect of resistance traits on seed shattering is not necessarily on the shattering genes per se, but most likely on the control of developmental genes and cell growth, effecting later maturity. Harboring these multiple resistance mechanisms could divert carbon flow into attaining enhanced xenobiotic detoxification or stress alleviation, sacrificing growth and development. In red rice (*Oryza sativa* L.), seed shattering is linked to particular genes, but these genes have not been linked to abiotic stress tolerance nor herbicide resistance (Akasaka et al. 2011).

Conclusions.

High resistance to propanil and quinclorac is common. There is intrapopulation difference in tesistance levels, with the majority of plants in these populations being highly resistant. Resistance to imazethapyr is less comment than to propanil or quinclorac in Arkansas; nevertheless, the resistance level to imazethapyr can be high. Resistance to cyhalofop is evolving. Multiple resistance is common. For example, Eco-45 has high levels of resistance to propanil and quinclorac, and low-level resistance to cyhalofop and elevated tolerance to glufosinate relative to other accessions. Populations with high resistance to propanil and quinclorac generally do not show fitness penalty. The absence of fitness penalties when growing under optimum conditions indicates that if these populations are left uncontrolled in a field the highly resistant biotypes will increase similarly to other biotypes in the soil seedbank.

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Tables

Table 3.1. Herbicide doses used in the dose response bioassay.

Treat-ment	Quinclorac		Propanil		Imazethapyr		Glufosinate		Cyhalofop	
	----------	----------	----------	----------	g ai ha^{-1} ----------	----------	----------	----------	----------	----------
1	0	(0)[a]	0	(0)[a]	0	(0)[a]	0	0.0 (0)[a]	0	(0)[a]
2	141	(1/4)	1121	(1/4)	20	(1/32)	18	(1/32)	18	(1/32)
3	282	(1/2)	2242	(1/2)	40	(1/16)	37	(1/16)	37	(1/16)
4	*565[b]	(1)	*4485[b]	(1)	80	(1/8)	74	(1/8)	74	(1/8)
5	1130	(2)	8971	(2)	*161[b]	(1/4)	*148[b]	(1/4)	*148[b]	(1/4)
6	2260	(4)	17942	(4)	322	(1/2)	297	(1/2)	297	(1/2)
7	3390	(6)	26913	(6)	644	(3/4)	445	(3/4)	445	(3/4)
8	4520	(8)	35884	(8)	1289	(1)	594	(1)	594	(1)
9	9041	(16)	71768	(16)	2579	(1.5)	891	(1.5)	891	(1.5)
10	18082	(32)	143536	(32)	--	(2)	1189	(2)	1189	(2)

[a] Numbers in parenthesis are the equivalent proportions of field rate of herbicide.
[b] *Indicates the rate used to isolate resistant and sensitive plants from parent populations.

Table 3.2. LD_{50} and I_{50} values calculated from regression models used to describe the relationship between plant injury and herbicide dose, and mortality and herbicide dose, using a 3- and 4-parameter sigmoid and low order polynomial models.

Accession[a]	Biotype[b]	Herbicide (1x rate g ai ha^{-1})	LD_{50}[c] (g ai ha^{-1})	I_{50}[d] (g ai ha^{-1})
Eco-26	S1	cyhalofop (594)	95	23
	S2	cyhalofop (594)	89	23
Eco-45	R1	cyhalofop (594)	297	231
	R2	cyhalofop (594)	297	166
Eco-208	R1	cyhalofop (594)	143	71
	R2	cyhalofop (594)	95	59
Eco-245	R1	cyhalofop (594)	219	118
	R2	cyhalofop (594)	172	65
Eco-45	R1	glufosinate (594)	162	148
	R2	glufosinate (594)	134	96
Eco-101	R1	imazethapyr (1289)	nc[e]	180
	R2	imazethapyr (1289)	nc[e]	322
Eco-225	R1	imazethapyr (1289)	nc[e]	386
	R2	imazethapyr (1289)	nc[e]	265
Eco-35	R1	propanil (4485)	4,350	67,275
	R2	propanil (4485)	3,991	22,873
Eco-45	R1	propanil (4485)	80,730	80,730
	R2	propanil (4485)	76,245	51,577
Eco-101	R1	propanil (4485)	53,820	10,091
	R2	propanil (4485)	44,850	19,509
Eco-225	R1	propanil (4485)	47,092	40,140
	R2	propanil (4485)	56,062	24,667
Eco-35	R1	quinclorac (565)	24,408	26,557
	R2	quinclorac (565)	791	186
Eco-45	R1	quinclorac (565)	nc[e]	nc[e]
	R2	quinclorac (565)	nc[e]	nc[e]
Eco-76	R1	quinclorac (565)	nc[e]	24,120
	R2	quinclorac (565)	nc[e]	10,170
Eco-101	R1	quinclorac (565)	904	502
	R2	quinclorac (565)	565	293
Eco-208	R1	quinclorac (565)	1921	932
	R2	quinclorac (565)	2881	841
Eco-245	R1	quinclorac (565)	2124	847
	R2	quinclorac (565)	5932	1243

[a] *E. colona* Accessions from Arkansas, USA collected between 2010-2015.

[b] Highly resistant (R1) or resistant (R2) biotypes; Sensitive (S1) and highly sensitive (S2) biotypes.

[c] LD_{50} is the estimated dose which kills 50% of the population.

[d] I_{50} is the estimated dose which causes 50% injury.

[e] nc = not controlled with the highest rate of herbicide.

Table 3.3. Observed injury (%) and survivors (%) at 0.5x and 2x rates of the herbicides used.

Acc[b]	Biotype[c]	Herbicide	Survivors 0.5x rate (%)	Injury 0.5x rate (%)	Survivors 2x rate (%)	Inury 2x rate (%)
Eco-26	S1	cyhalofop	0 (0)	99 (0)	0 (0)	99 (0)
	S2	cyhalofop	0 (0)	100 (0)	0.0 (0)	100 (0)
Eco-45	R1	cyhalofop	49 (7)	78 (2)	16 (5)	96 (2)
	R2	cyhalofop	54 (10)	67 (10)	4 (10)	99 (10)
Eco-208	R1	cyhalofop	8 (5)	99 (2)	0 (5)	100 (0)
	R2	cyhalofop	0 (5)	100 (4)	0 (5)	100 (4)
Eco-245	R1	cyhalofop	0 (4)	100 (4)	0 (4)	100 (4)
	R2	cyhalofop	0 (7)	100 (5)	0 (7)	100 (5)
Eco-45	R1	glufosinate	96 (4)	14 (1)	0 (4)	100 (0)
	R2	glufosinate	78 (10)	30 (8)	0 (8)	100 (8)
Eco-101	R1	imazethapyr	nc[d]	17 (0)	nc[d]	30 (6)
	R2	imazethapyr	nc[d]	26 (5)	nc[d]	55 (3)
Eco-225	R1	imazethapyr	nc[d]	27 (0)	nc[d]	45 (0)
	R2	imazethapyr	nc[d]	6 (0)	nc[d]	19 (5)
Eco-35	R1	propanil	47 (13)	2 (1)	38 (14)	26 (1)
	R2	propanil	42 (13)	15 (3)	32 (14)	32 (3)
Eco-45	R1	propanil	100 (4)	0 (1)	100 (8)	6 (2)
	R2	propanil	95 (8)	0 (0)	92 (9)	16 (3)
Eco-101	R1	propanil	83 (11)	10 (4)	85 (15)	50 (2)
	R2	propanil	100 (9)	0 (5)	100 (9)	20 (5)
Eco-225	R1	propanil	100 (10)	4 (3)	83 (10)	24 (3)
	R2	propanil	99 (10)	12 (7)	100 (8)	22 (5)
Eco-35	R1	quinclorac	100 (3)	0 (0)	100 (3)	3 (0)
	R2	quinclorac	100 (4)	87 (0)	25 (4)	98 (0)
Eco-45	R1	quinclorac	nc[d]	2 (2)	nc[d]	15 (2)
	R2	quinclorac	nc[d]	2 (2)	nc[d]	9 (2)
Eco-76	R1	quinclorac	100 (1)	0 (4)	100 (1)	10 (3)
	R2	quinclorac	100 (2)	0 (2)	100 (2)	8 (1)
Eco-101	R1	quinclorac	100 (5)	39 (1)	16 (5)	99 (1)
	R2	quinclorac	100 (48)	46 (0)	25 (48)	100 (0)
Eco-208	R1	quinclorac	100 (8)	5 (1)	84 (8)	84 (1)
	R2	quinclorac	100 (6)	6 (1)	75 (6)	87 (3)
Eco-245	R1	quinclorac	100 (8)	5 (4)	100 (8)	34 (4)
	R2	quinclorac	100 (8)	5 (1)	100 (8)	40 (1)

[a] Numbers in parenthesis are the standard errors.

[b] *E. colona* accessions gathered from Arkansas, USA from 2010-2015.

[c] Highly resistant (R1) or resistant (R2) biotypes; sensitive (S1) and highly sensitive (S2) biotypes.

[d] nc = no control from highest rate of herbicide.

Table 3.4. Characterization of selected accessions with confirmed resistance to various herbicides.

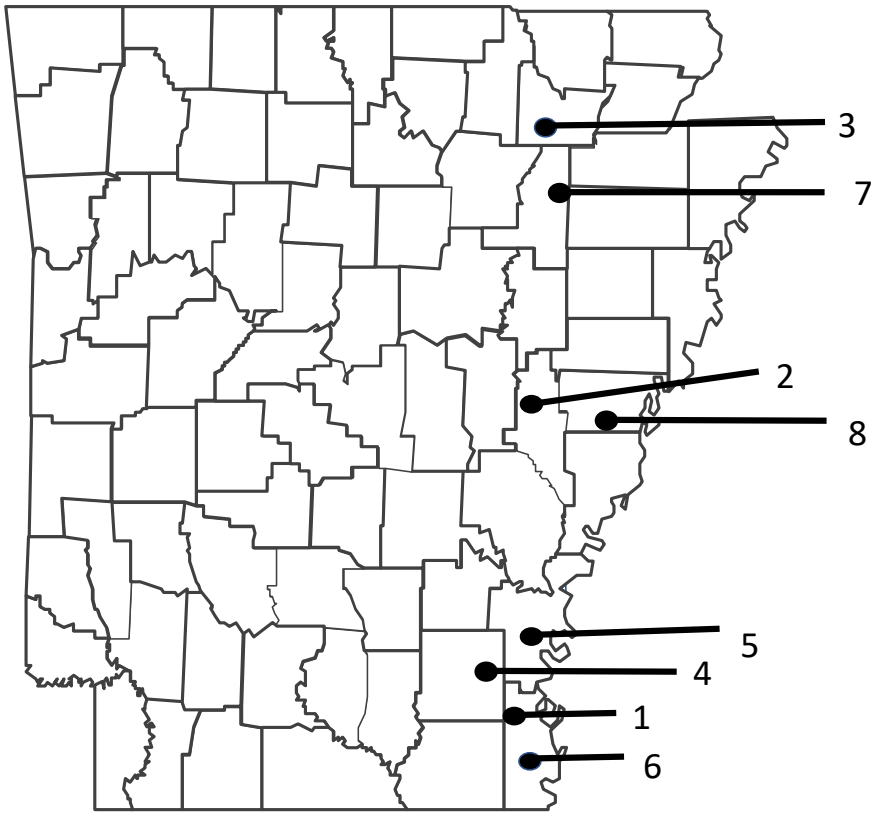
Accession[a]	Biotype[b]	Tiller height[c]	Days to seed shattering[c]	Seeds per panicle[c]	1000 ct. seed weight[c]
		(cm)	(#)	(#)	(g)
Eco-45 (P,Q)	R1	108	96	167	1.5 b
	R2	109	98	328	1.9 a
Eco-101 (P,Q,I)	R1	86	99	214	2.4
	R2	95	96	343	2.3
Eco-225 (P, I)	R1	70	104	359	2.5
	R2	69	92	237	2.3
Eco-35 (P,Q)	R1	111	104	396 a	2.2
	R2	109	95	251 b	2.3
Eco-208 (Q)	R1	96	110 a	269	2.2
	R2	95	96 b	290	2.4
Eco-245 (Q)	R1	83	110	213	2.4
	R2	79	96	314	2.4
Eco-26	S1	83	104	94	2.4
	S2	84	102	213	2.4
Eco-76 (Q)	R1	125	96	366	1.9
	R2	118	96	388	2.3

[a] *E. colona* accessions gathered from Arkansas, USA between 2010 and 2015; P= propanil, Q= quinclorac, C= cyhalofop, I =imazethapyr, G =glufosinate.

[b] Highly resistant (R1) or resistant (R2) biotypes; sensitive (S1) and highly sensitive (S2) biotypes isolated from field populations in 2015 by spraying sublethal rates of pertinent herbicides at the 4-leaf stage.

[c]R1 and R2 mean pairs of each accession without letters are not different using Fischer's protected LSD (α=0.05).

Figures



	Accession	Resistance Profile
1	Eco-26	cyhalofop
2	Eco-35	Propanil, quinclorac
3	Eco-45	Propanil, quinclorac, glufosinate, cyhalofop
4	Eco-76	Quinclorac
5	Eco-101	Propanil, quinclorac, imazethapyr
6	Eco-208	Quinclorac, cyhalofop
7	Eco-225	Propanil, imazethapyr
8	Eco-245	Quinclorac, cyhalofop

Figure 3.1. Resistance profiles and origin of samples.

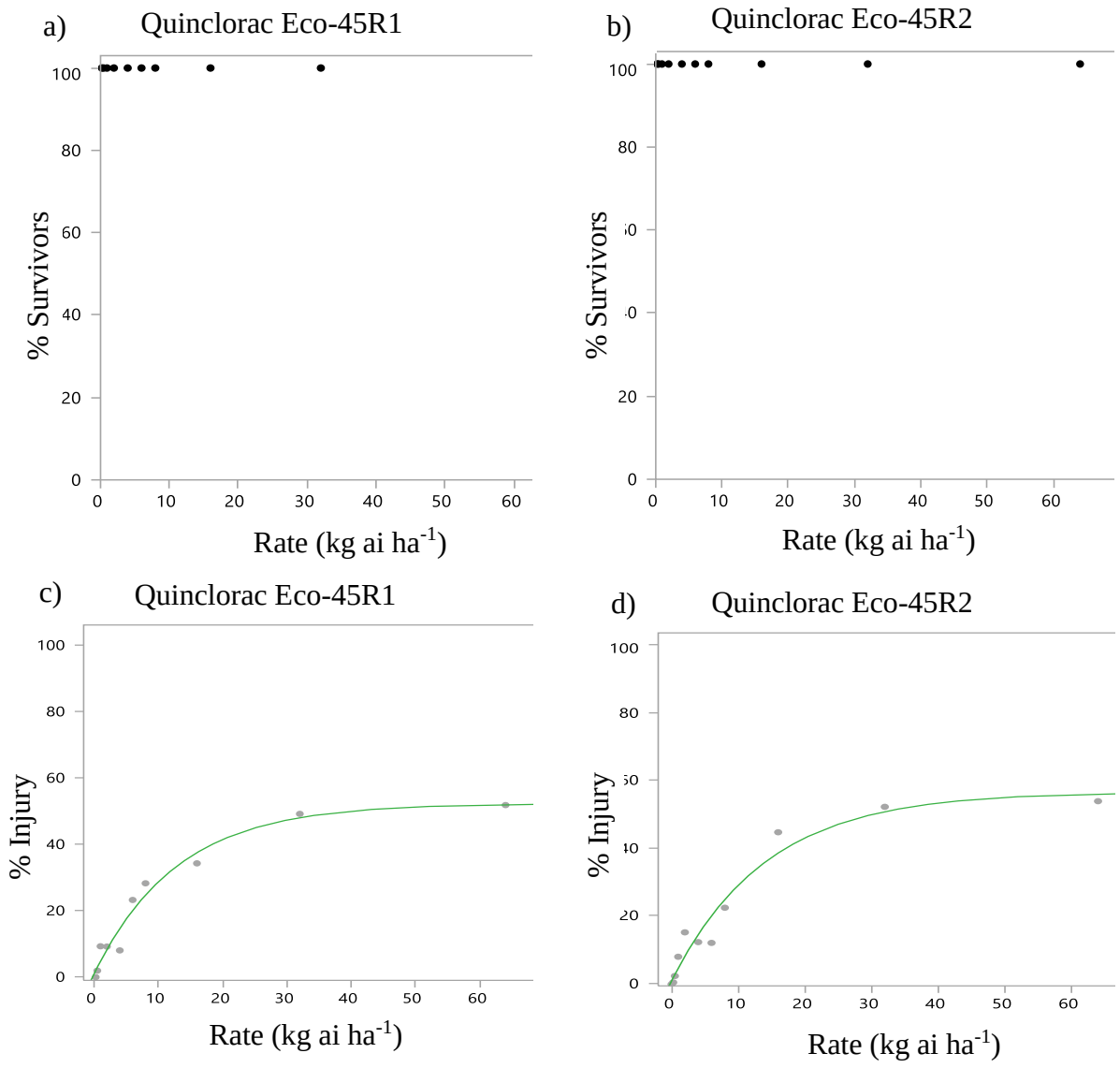


Figure 3.2. *Echinochloa colona* survivors (%) from accession Eco-45R1 and R2 (a-b) and level of injury of survivors (c-d) following equation y= a+b*EXP(c*rate) from Arkansas, USA across rates of quinclorac.

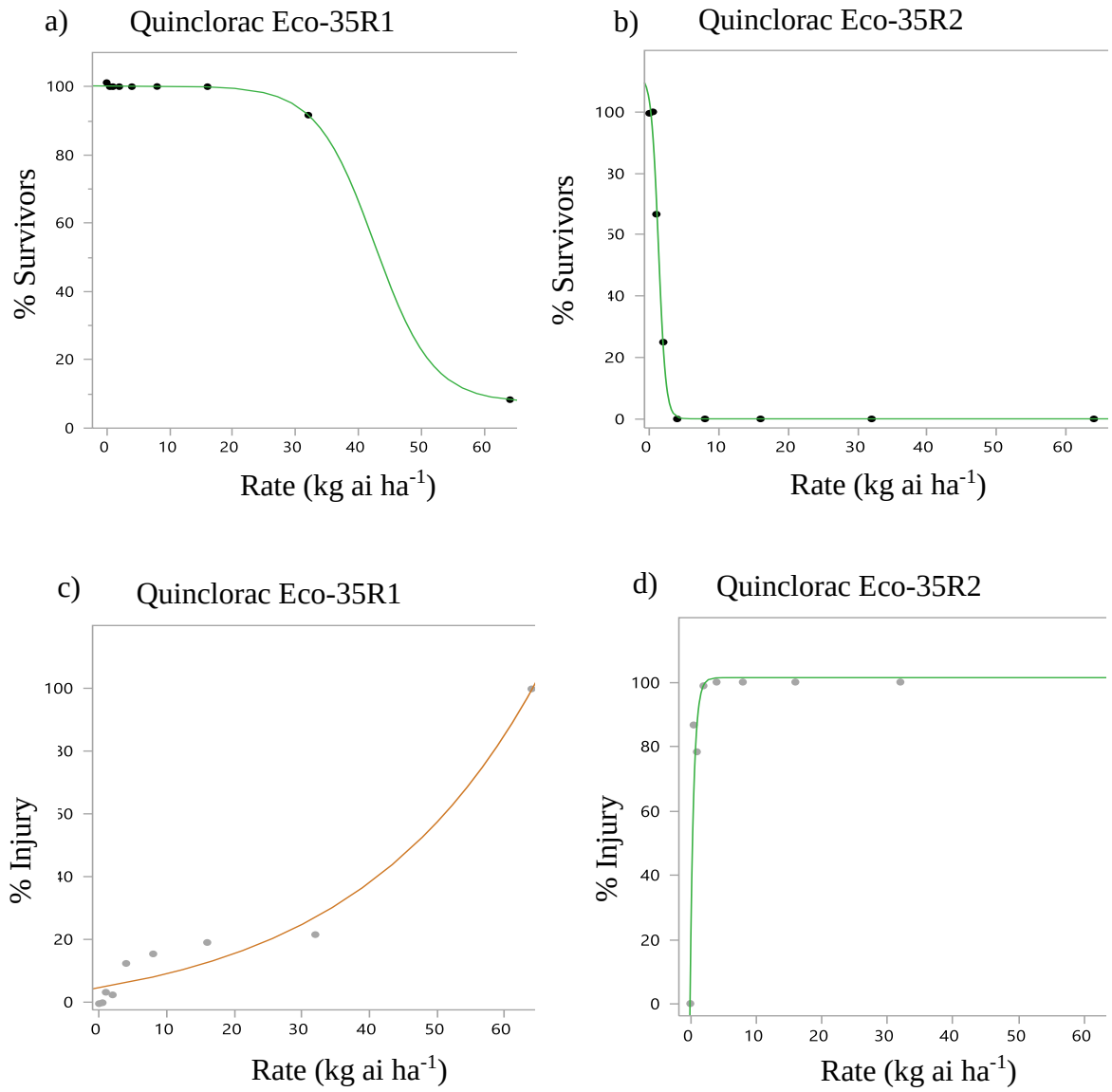
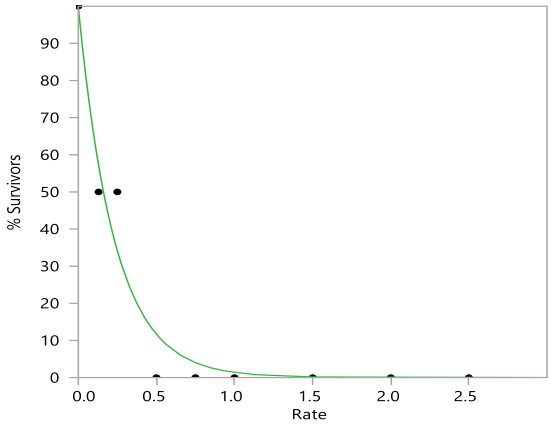


Figure 3.3 Survivors (%) of Eco-35R1 and R2 (a-b) following model y= c+((d-c)/(1+EXP(-a(rate-b)))) and level of injury of survivors (c-d) following model y= a+b*EXP(c*rate) and y= c+((d-c)/(1+EXP(-a(rate-b)))) from Arkansas, USA across different rates of quinclorac.

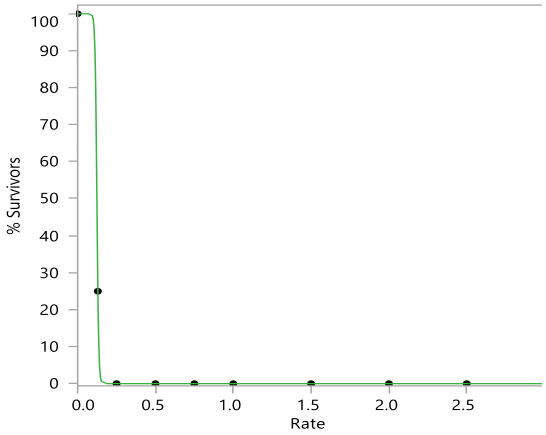
Appendix

Appendix A- Survivors (%) of Eco-26, 45, and 208 from Arkansas, USA across different rates of Cyhalofop.

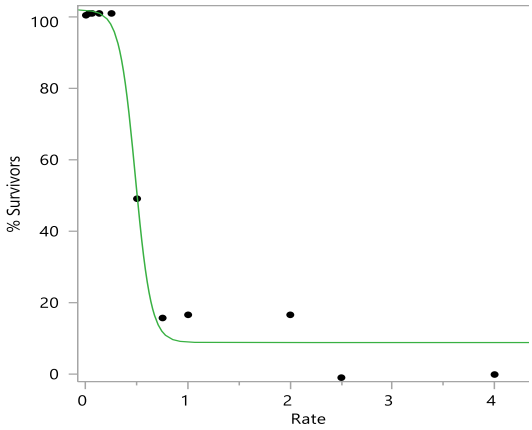
Survivors (%) Cyhalofop Eco-26S1



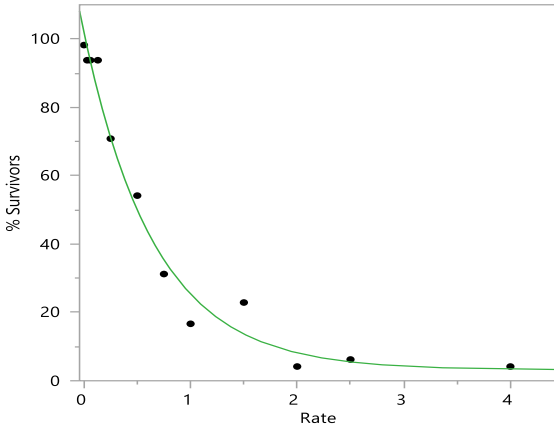
Survivors (%) Cyhalofop Eco-26S2



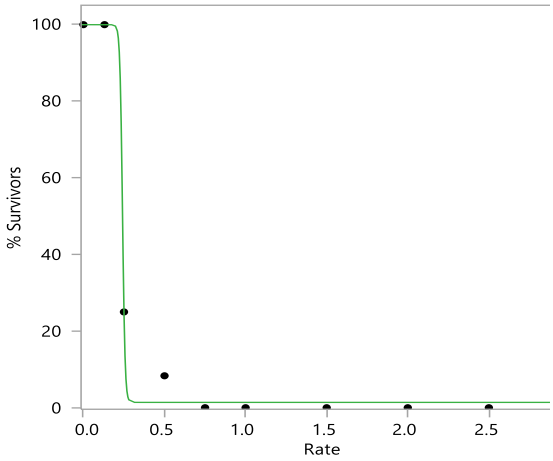
Survivors (%) Cyhalofop Eco-45R1



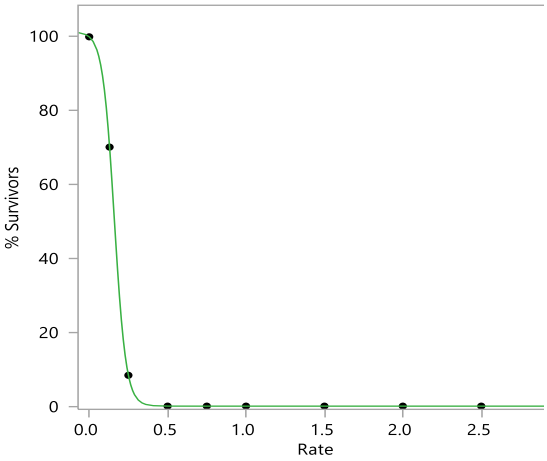
Survivors (%) Cyhalofop Eco-45R2



Survivors (%) Cyhalofop Eco-208R1

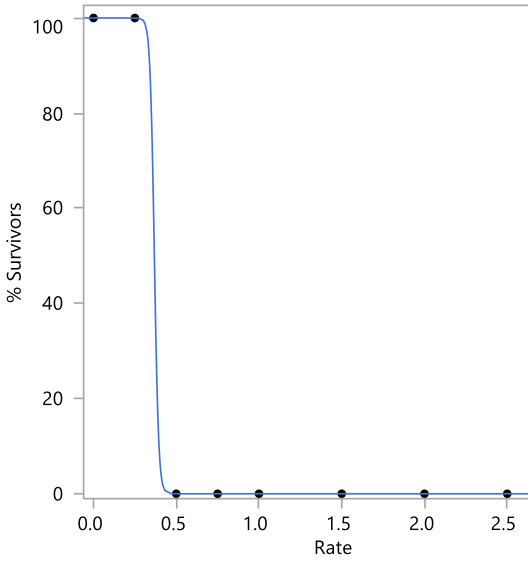


Survivors (%) Cyhalofop Eco-208R2

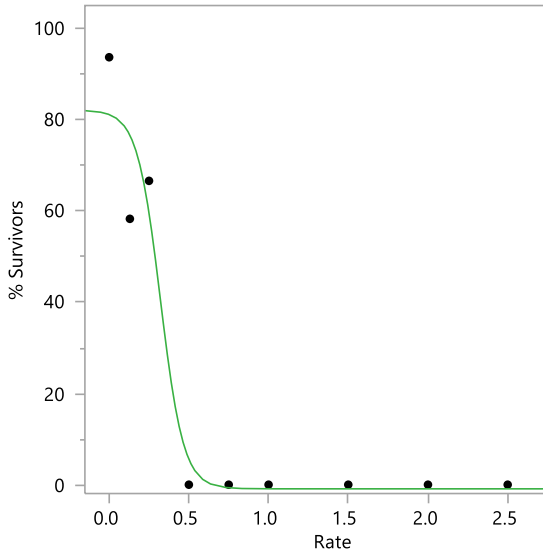


Appendix B- Survivors (%) of Eco-245 and 45 from Arkansas, USA across different rates of cyhalofop and glufosinate.

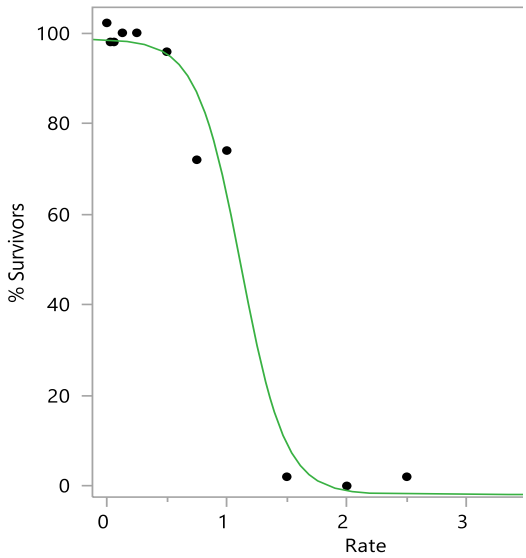
Survivors (%) Cyhalofop Eco-245R1



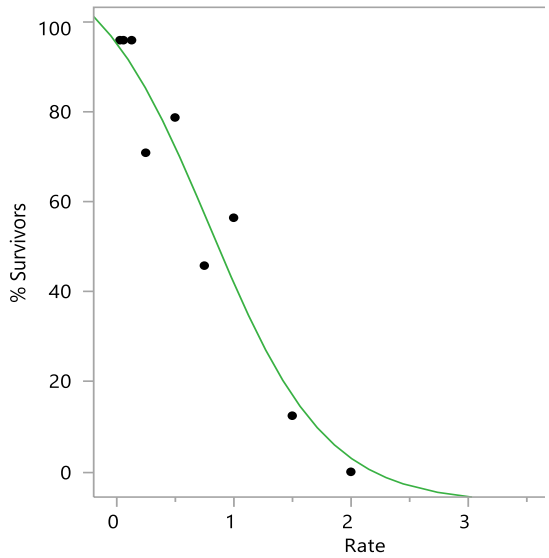
Survivors (%) Cyhalofop Eco-245R2



Survivors (%) Glufosinate Eco-45R1

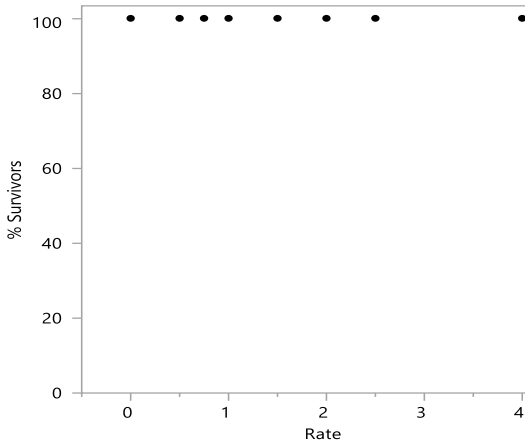


Survivors (%) Glufosinate Eco-45R2

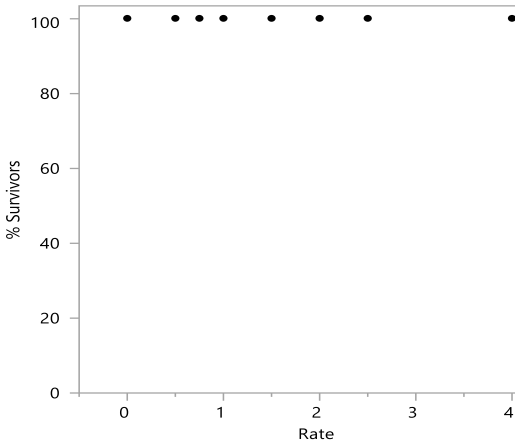


Appendix C- Survivors (%) of Eco-101 and 225 from Arkansas, USA across different rates of imazethapyr.

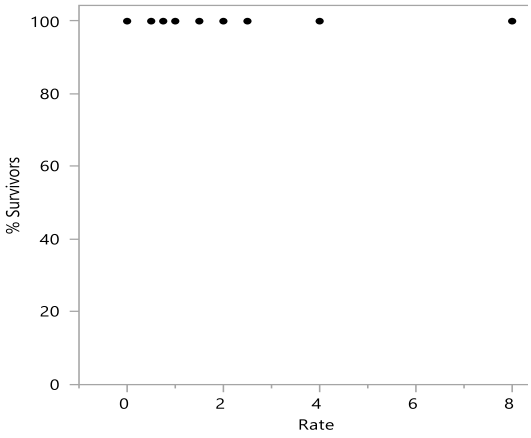
Survivors (%) Imazethapyr Eco-101R1



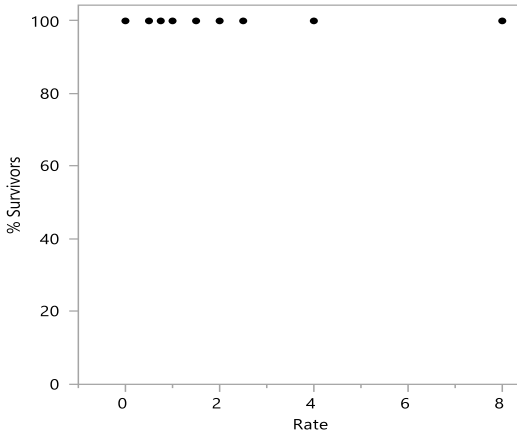
Survivors (%) Imazethapyr Eco-101R2



Survivors (%) Imazethapyr Eco-225R1

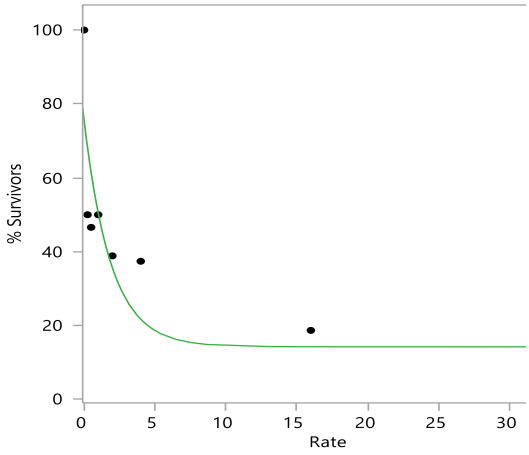


Survivors (%) Imazethapyr Eco- 225R2

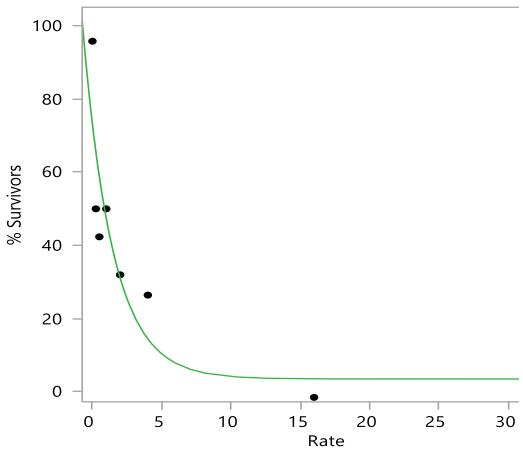


Appendix D - Survivors (%) of Eco-35, 45, and 101 from Arkansas, USA across different rates of propanil.

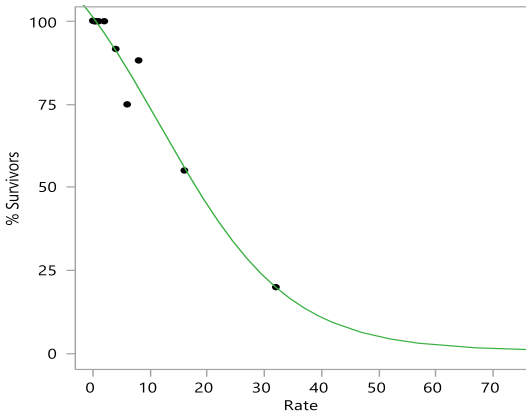
Survivors (%) Propanil Eco-35R1



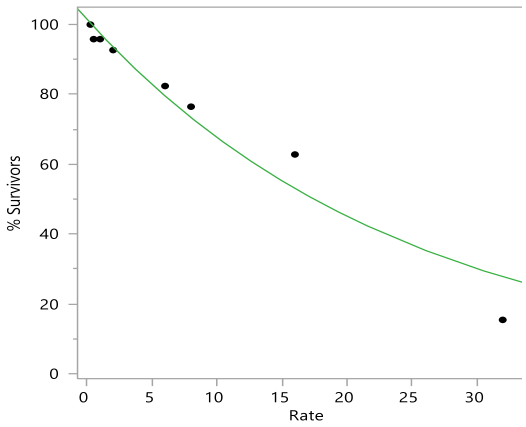
Survivors (%) Propanil Eco-35R2



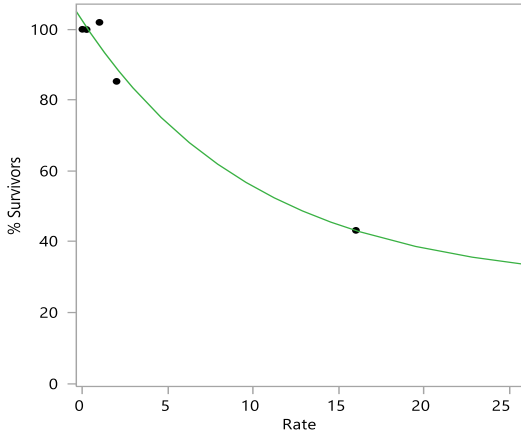
Survivors (%) Propanil Eco-45R1



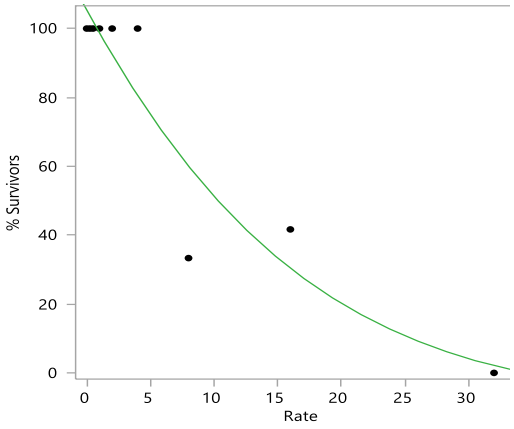
Survivors (%) Propanil Eco-45R2



Survivors (%) Propanil Eco-101R1

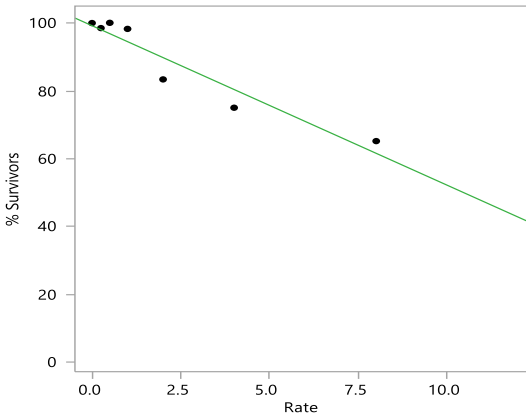


Survivors (%) Propanil Eco- 101R2

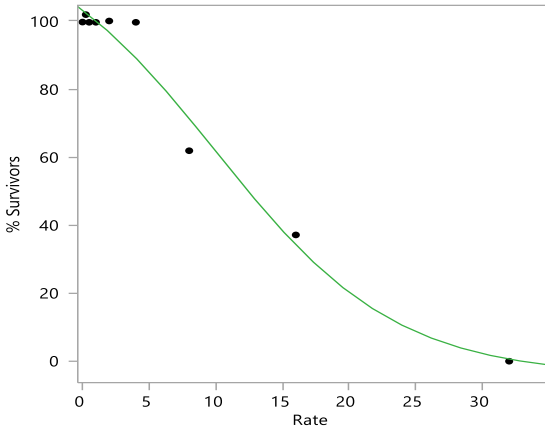


Appendix E- Survivors (%) for Eco-225 from Arkansas, USA across different rates of propanil.

Survivors (%) Propanil Eco- 225R1

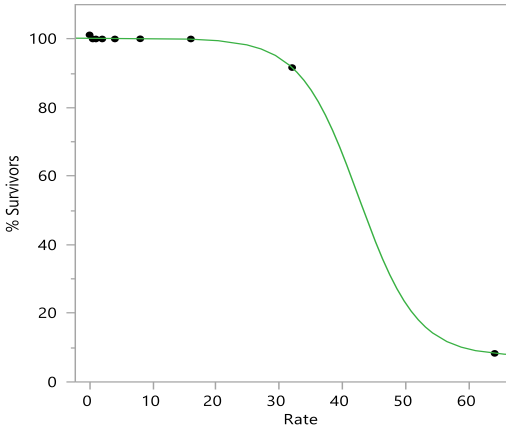


Survivors (%) Propanil Eco-225R2

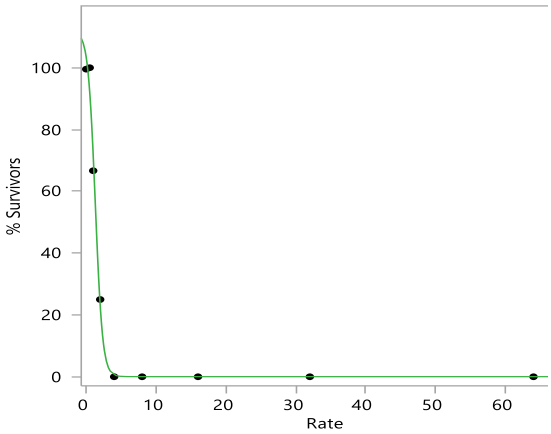


Appendix F- Survivors (%) of Eco-35, 45, and 76 from Arkansas, USA across different rates of quinclorac.

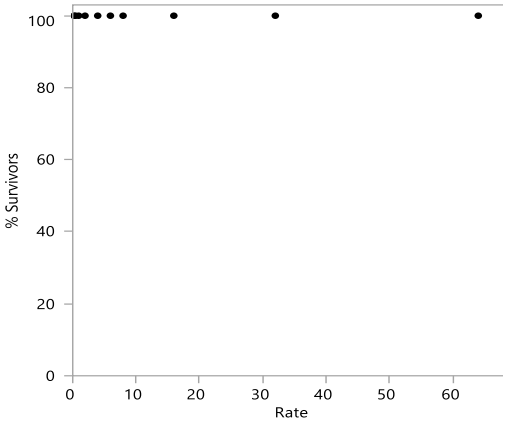
Survivors (%) Quinclorac Eco-35R1



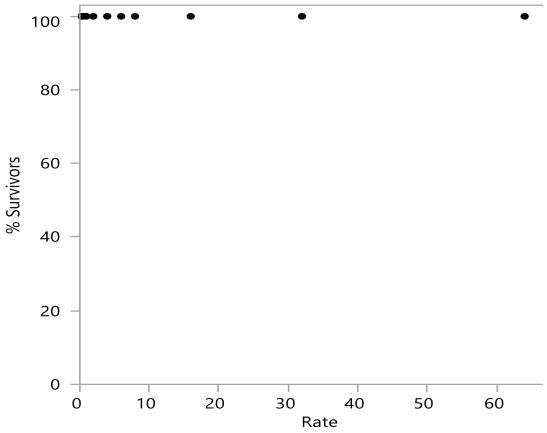
Survivors (%) Quinclorac Eco-35R2



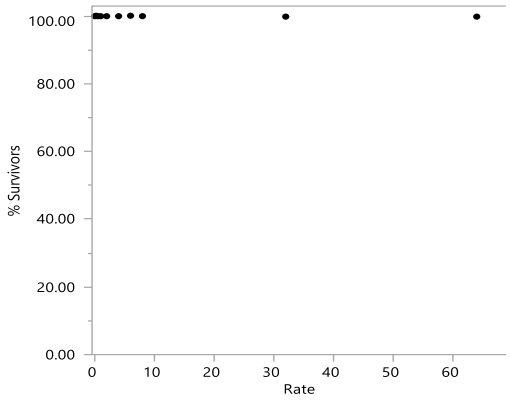
Survivors (%) Quinclorac Eco-45R1



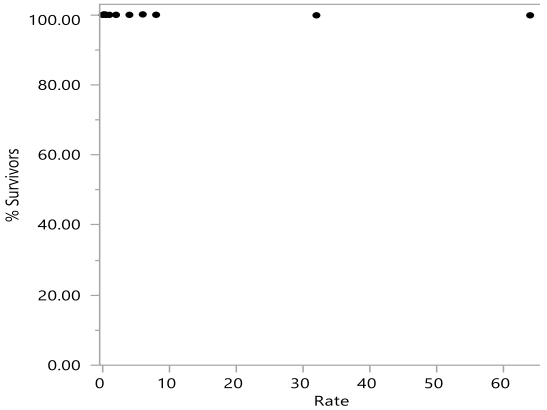
Survivors (%) Quinclorac Eco-45R2



Survivors (%) Quinclorac Eco-76R1

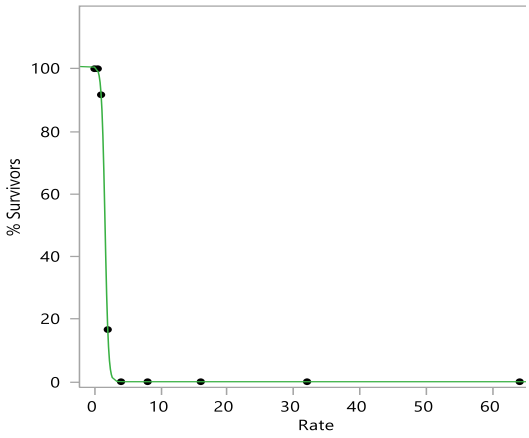


Survivors (%) Quinclorac Eco-76R2

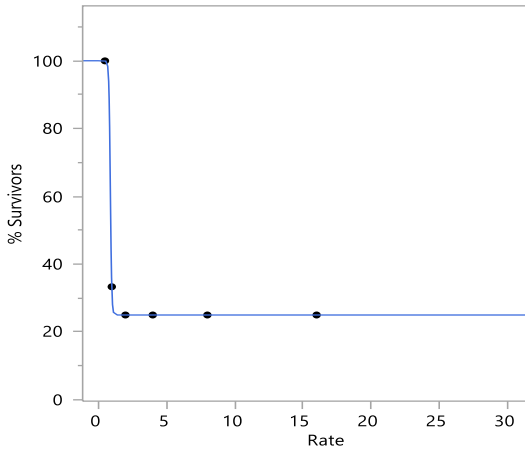


Appendix G-Survivors (%) of Eco-101, 208, and 245 from Arkansas, USA across different rates of quinclorac.

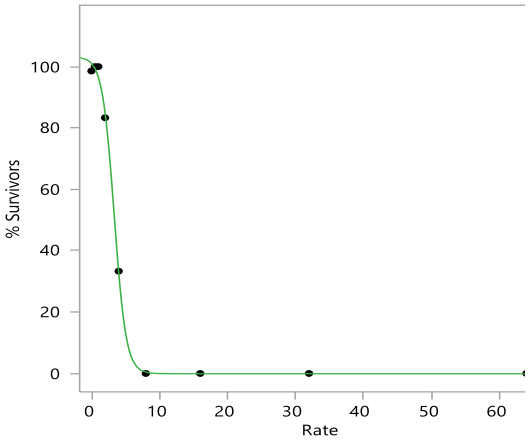
Survivors (%) Quinclorac Eco-101R1



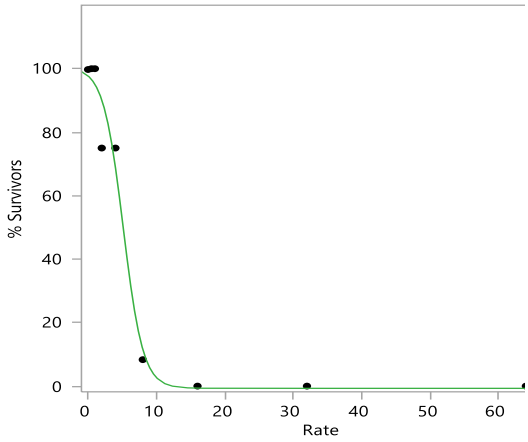
Survivors (%) Quinclorac Eco-101R2



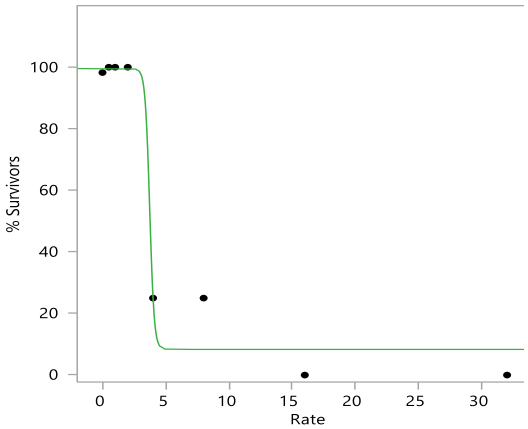
Survivors (%) Quinclorac Eco-208R1



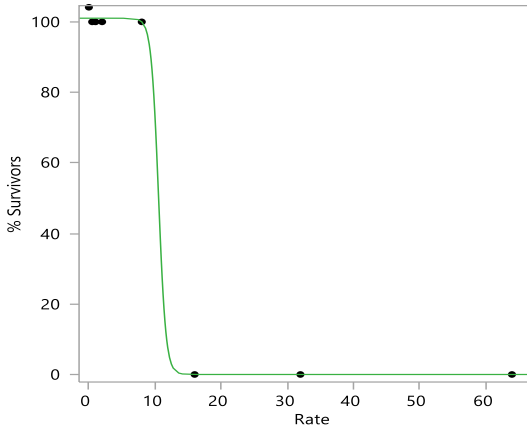
Survivors (%) Quinclorac Eco-208R2



Survivors (%) Quinclorac Eco-245R1

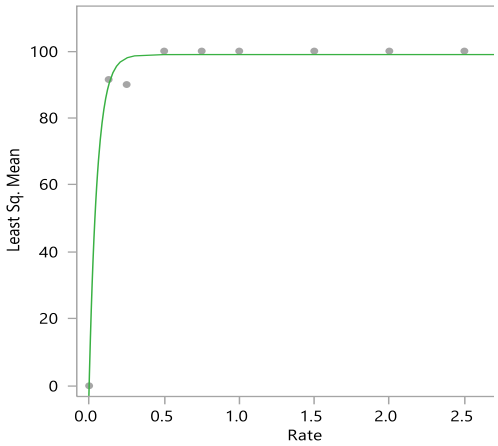


Survivors (%) Quinclorac Eco-245R2

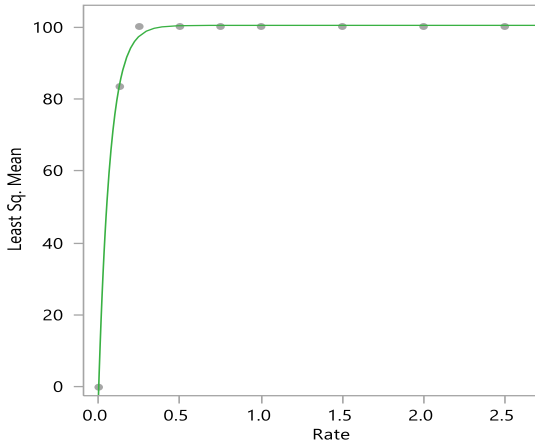


Appendix H- Injury (%) of Eco-26, 45, 208 from Arkansas, USA across different rates of cyhalofop.

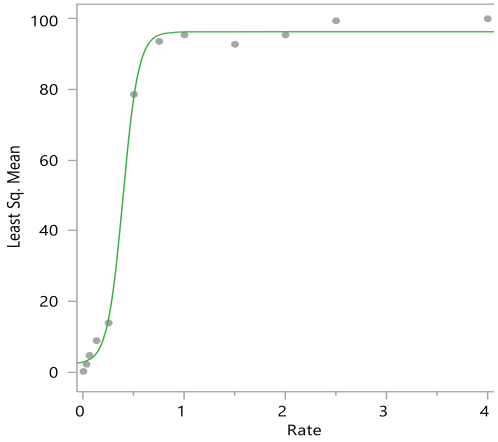
Injury (%) Cyhalofop Eco-26S1



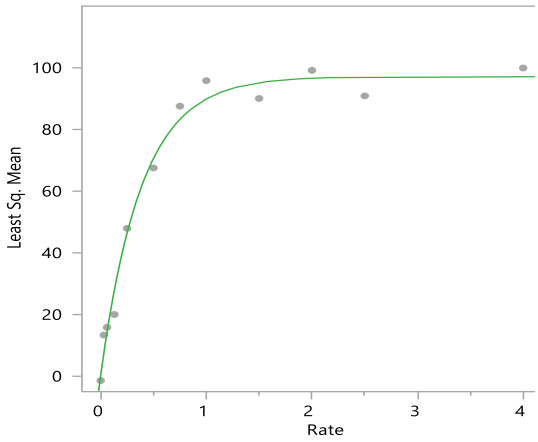
Injury (%) Cyhalofop Eco-26S2



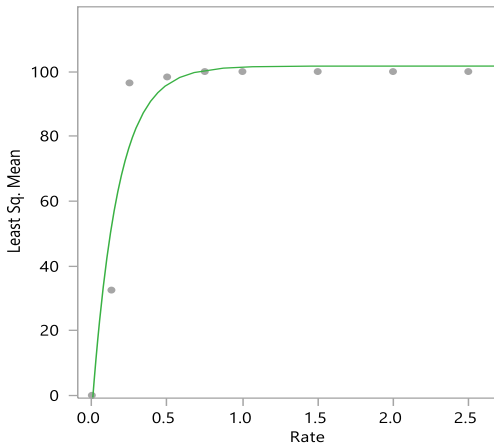
Injury (%) Cyhalofop Eco-45R1



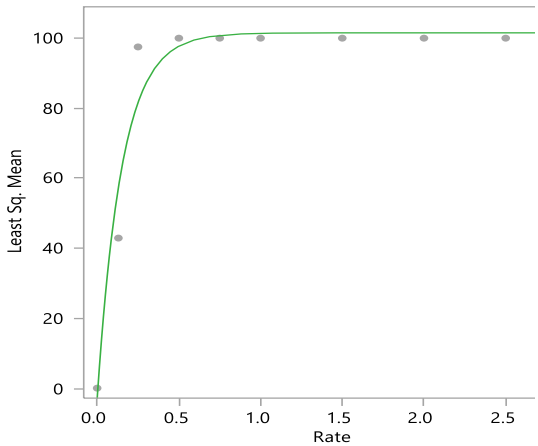
Injury (%) Cyhalofop Eco-45R2



Injury (%) Cyhalofop Eco-208R1

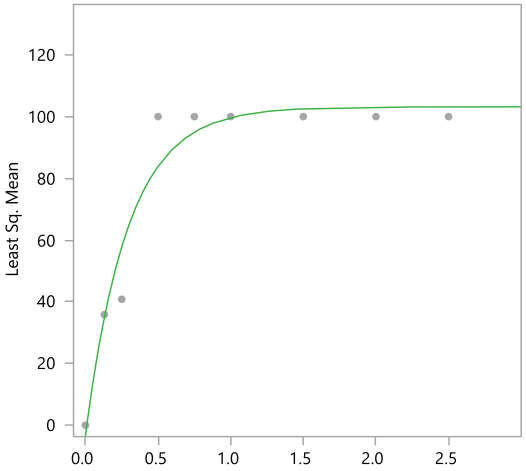


Injury (%) Cyhalofop Eco-208R2

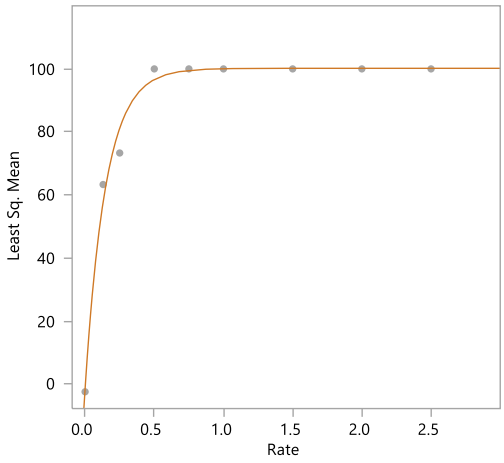


Appendix I- Injury (%) of Eco-245 and 45 from Arkansas, USA across different rates of cyhalofop and glufosinate.

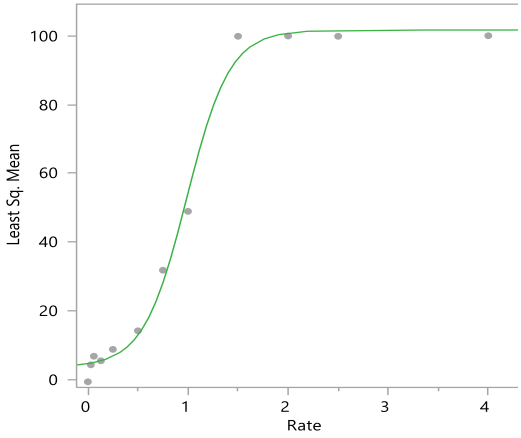
Injury (%) Cyhalofop Eco-245-R1



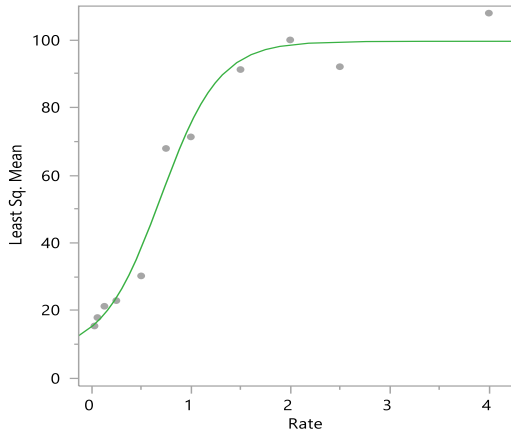
Injury (%) Cyhalofop Eco-245-R2



Injury (%) Glufosinate Eco-45-R1

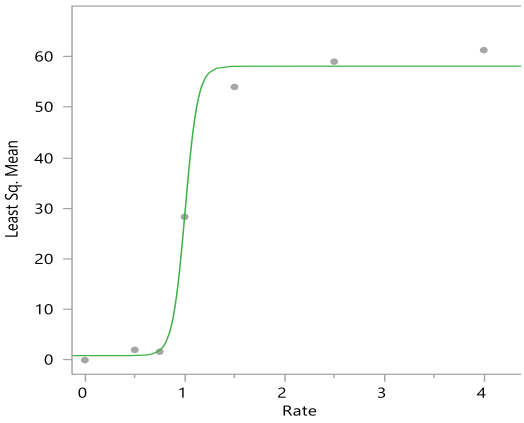


Injury (%) Glufosinate Eco-45-R2

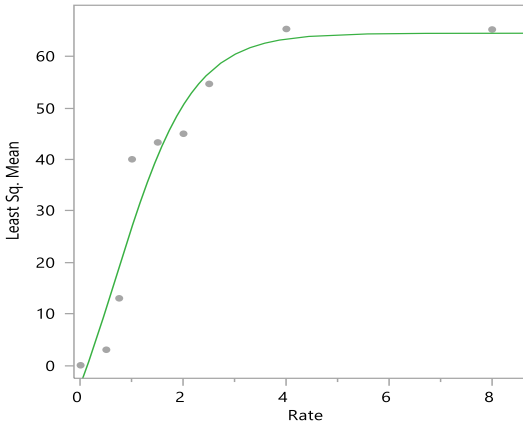


Appendix J- Injury (%) of Eco-101 and 225 from Arkansas, USA across different imazethapyr rates.

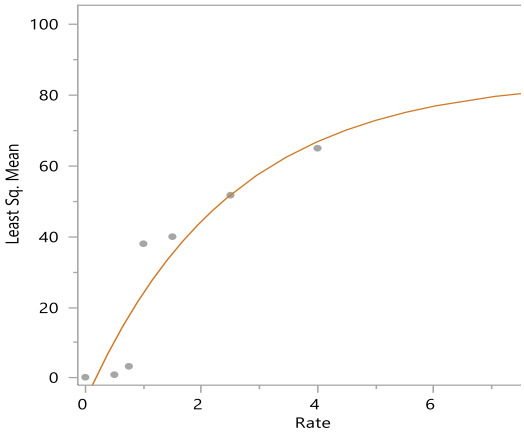
Injury (%) Imazethapyr Eco-101-R1



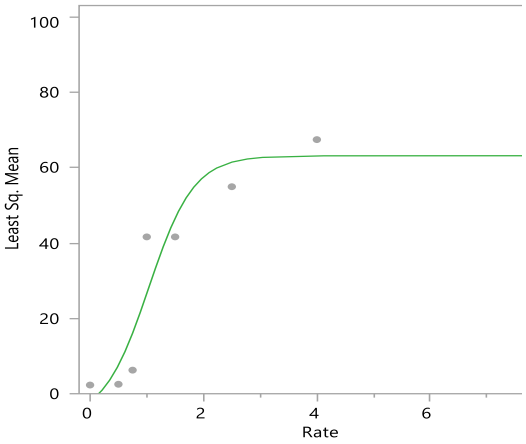
Injury (%) Imazethapyr Eco-101-R2



Injury (%) Imazethapyr Eco-225-R1

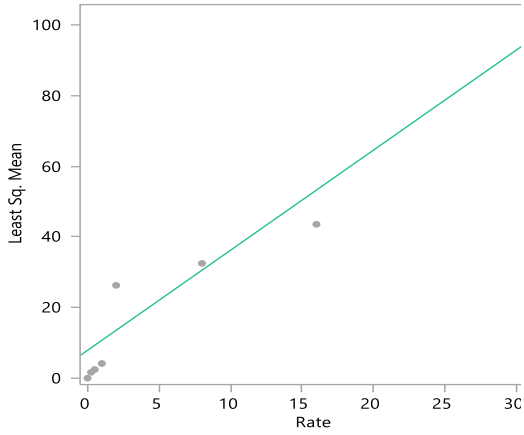


Injury (%) Imazethapyr Eco-225-R2

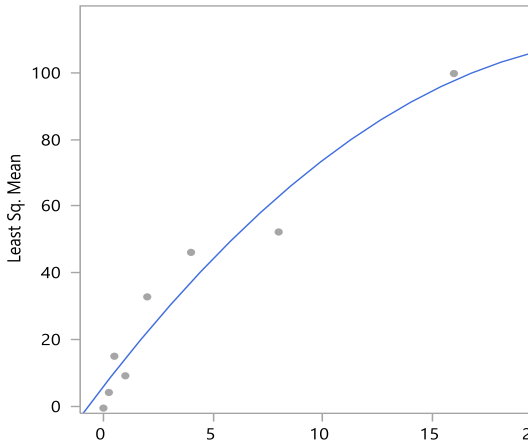


Appendix K- Injury (%) of Eco-35, 45, and 101 from Arkansas, USA across different propanil rates.

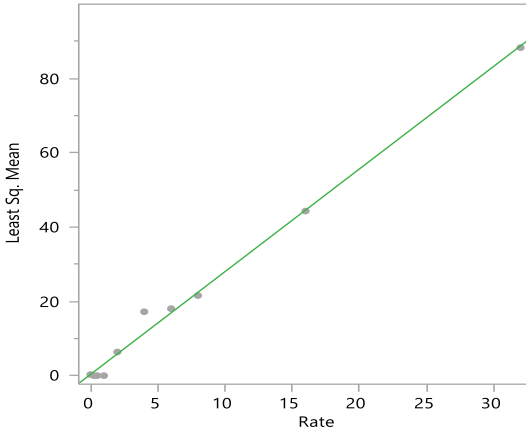
Injury (%) Propanil Eco-35-R1



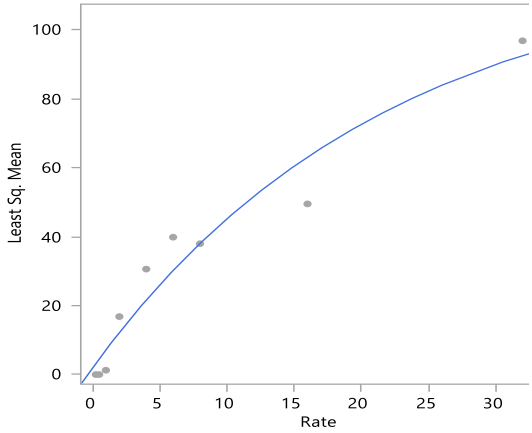
Injury (%) Propanil Eco-35-R2



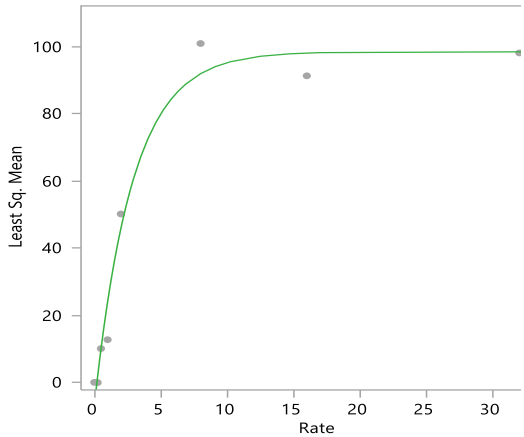
Injury (%) Propanil Eco-45-R1



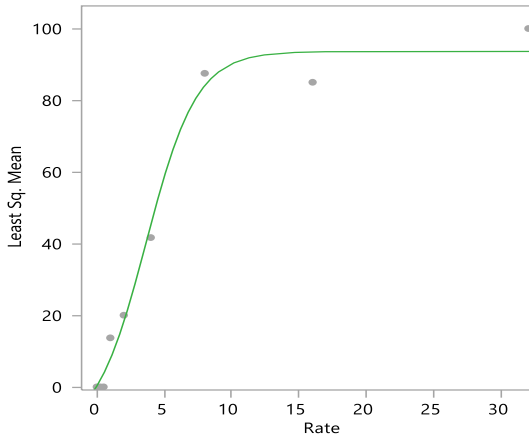
Injury (%) Propanil Eco-45-R2



Injury (%) Propanil Eco-101-R1

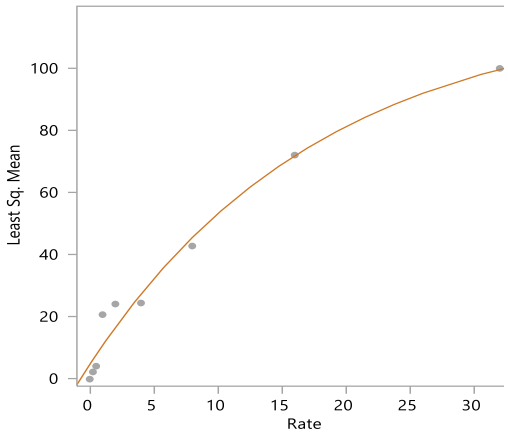


Injury (%) Propanil Eco-101-R2

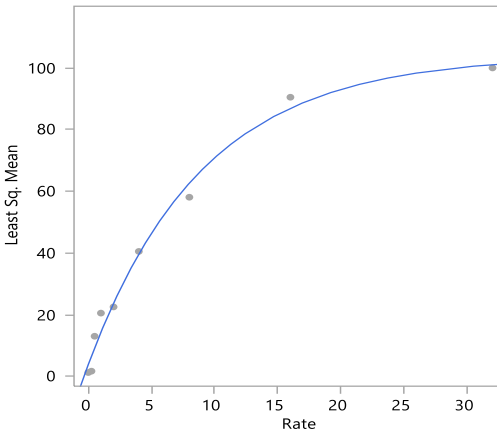


Appendix L- Injury (%) of Eco-225 from Arkansas, USA from different Propanil rates.

Injury (%) Propanil Eco-225-R1

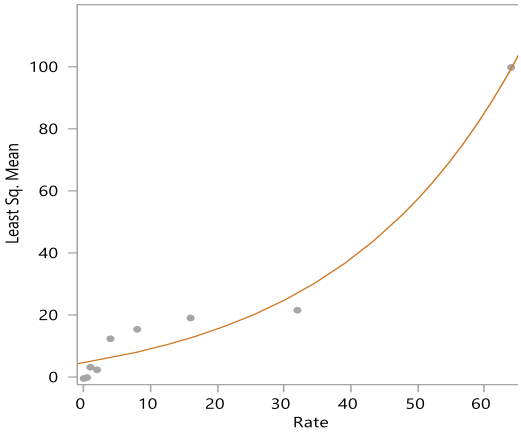


Injury (%) Propanil Eco-225-R2

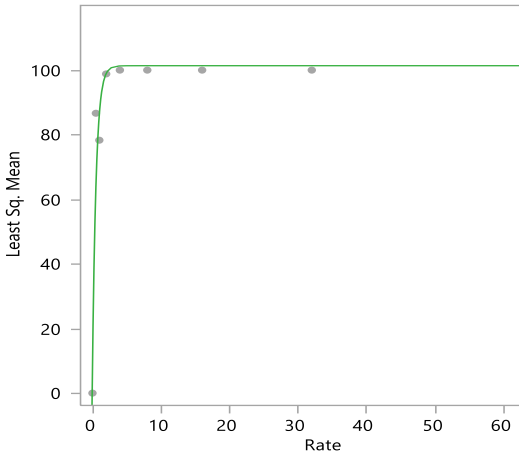


Appendix M- Injury (%) of Eco-35, 45, and 76 from Arkansas, USA across different quinclorac rates.

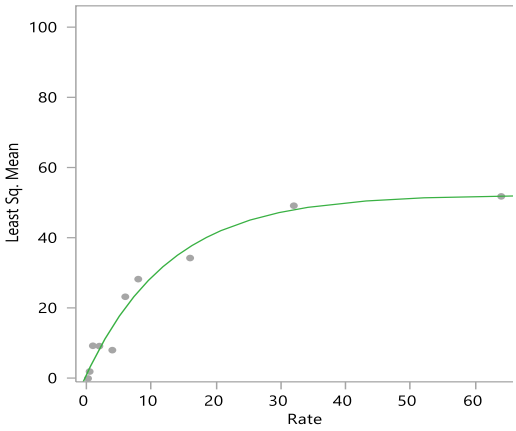
Injury (%) Quinclorac Eco-35-R1



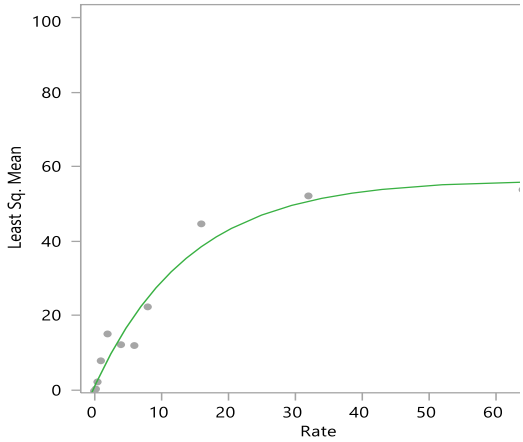
Injury (%) Quinclorac Eco-35-R2



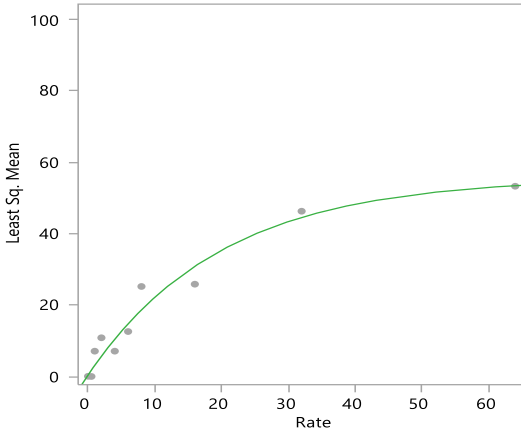
Injury (%) Quinclorac Eco-45-R1



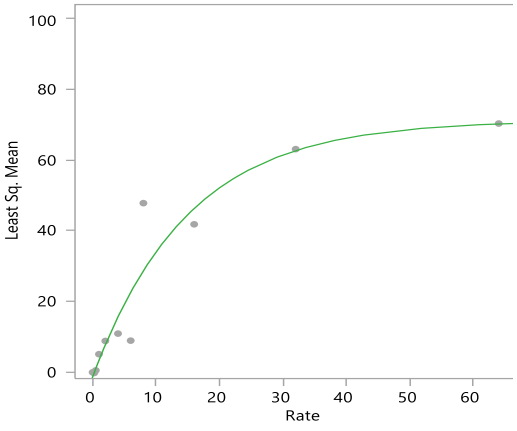
Injury (%) Quinclorac Eco-45-R2



Injury (%) Quinclorac Eco-76-R1

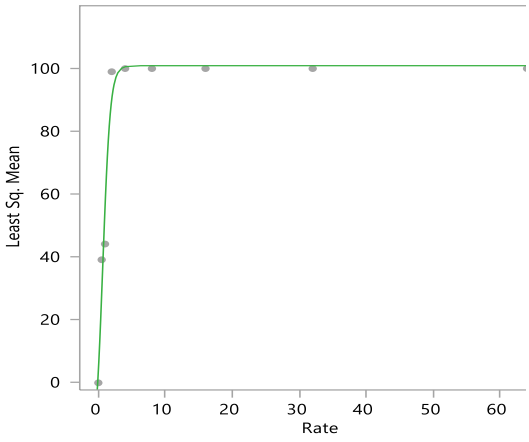


Injury (%) Quinclorac Eco-76-R2

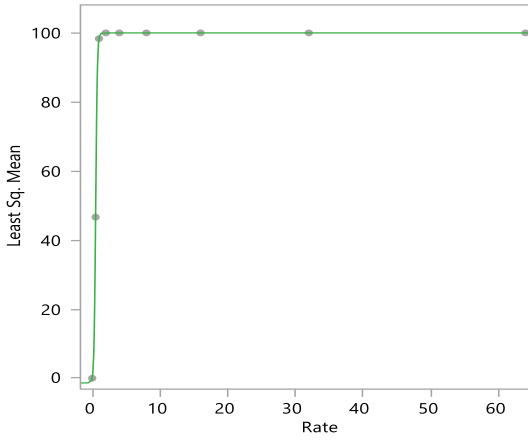


Appendix N- Injury (%) of Eco-101, 208, and 245 from Arkansas, USA for different quinclorac rates.

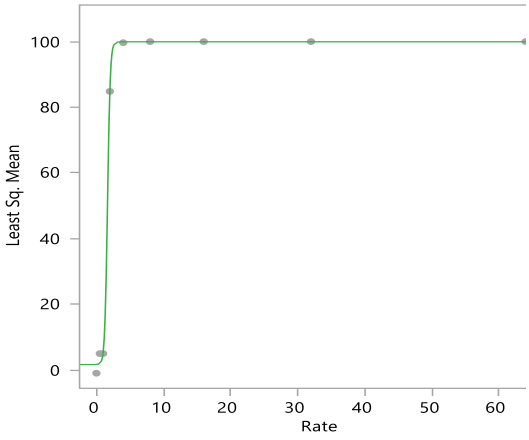
Injury (%) Quinclorac Eco-101-R1



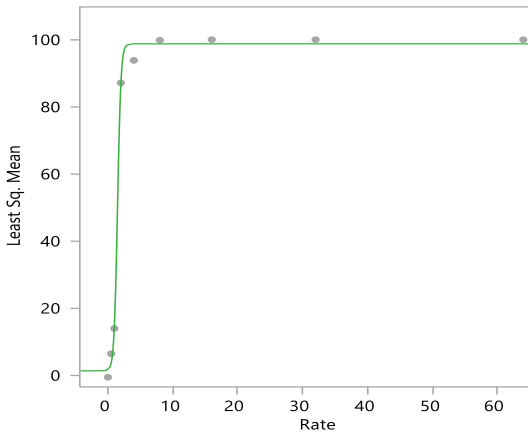
Injury (%) Quinclorac Eco-101-R2



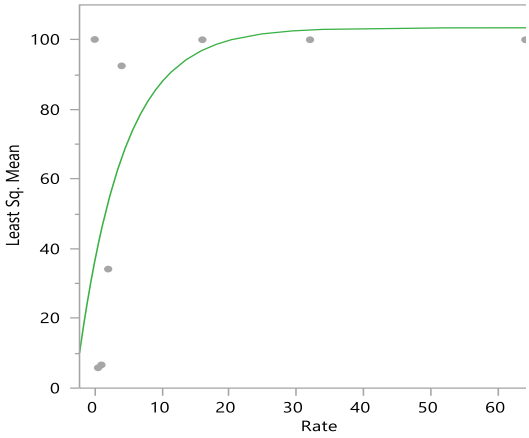
Injury (%) Quinclorac Eco-208-R1



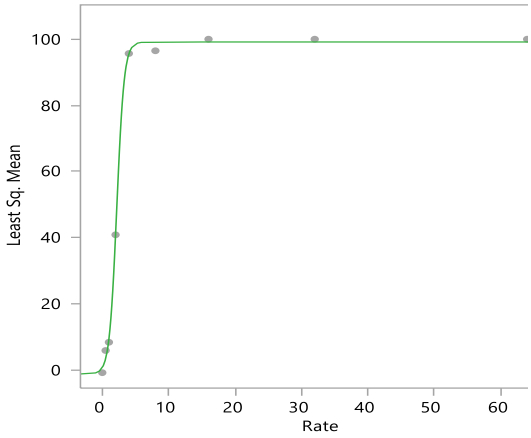
Injury (%) Quinclorac Eco-208-R2



Injury (%) Quinclorac Eco-245-R1



Injury (%) Quinclorac Eco-245-R2



Chapter 4: Competitive Ability of Herbicide-Resistant *Echinochloa colona* with Rice

Abstract

Replacement series experiments are utilized to understand interference between species or cultivars of the same species. Replacement studies can elucidate the effect of herbicide resistance traits on the competitive ability of resistant weed populations. The objectives of this research were to determine if single- or multiple-herbicide-resistant ecotypes of junglerice (*Echinochloa colona*) (L.) Link) differ in competitive ability and if certain herbicide resistance traits are more likely to reduce weed-competitive ability. Eight accessions with different resistance profiles were compared in a 1:1 competition study. A replacement series experiment was also conducted, which included biotypes of Eco-76 (resistant to quinclorac only) and Eco-45 (highly resistant to quinclorac and propanil, with low-level resistance to cyhalofop and elevated tolerance to glufosinate).The quinclorac-resistant Eco-76 was the tallest (125 cm) and largest plant (64 g plant^{-1}). Eco-225, with resistance to propanil and imazethapyr, was the shortest (69 cm) and smallest plant (51 g plant^{-1}). In the 1:1 competition study, highly resistant (R1) and resistant (R2) biotypes of all accessions competed equally with rice (*Oryza sativa* (L.) Beauv). Eco-26, which was susceptible to all herbicides, was one of the shortest and smallest plants. In the replacement series experiment, Eco-76 competed more with rice than did Eco-45, regardless of biotype. The competitive ratio, aggressiveness index, and relative crowding coefficient of both Eco-45 and Eco-76 indicate that both resistant ecotypes were aggressive competitors with rice. Eco-76 grew taller and produced more biomass per plant when grown in mixtures with rice compared to the monoculture control. The replacement series study showed that Eco-76 was a stronger competitor with rice than Eco-45. Intraspecific competition among junglerice plants was stronger than interspecific competition with rice. Extreme high resistance to propanil and quinclorac had no effect on the competitive ability of junglerice.

Introduction

Rice ranks amongst the top three most important grain crops worldwide, and is a staple food for approximately 50% of the world population (Chauhan et al. 2017). About 600,000 ha of rice was harvested in 2016 in Arkansas (Hardke et al. 2017), the largest producer of rice in the United States. Weed competition is a major constraint in rice production, requiring the highest production input to ensure optimum yield. The major weed species that impact rice include grasslike fimbristylis (*Fimbristylis miliacea* (L.) Vahl), seedbox (*Ludwigia hyssopifolia* (G. Don) Exell), and flatsedge (*Cyperus difformis* L.). Of the many weed species in rice, *Echinochloa spp.* are the most widely observed and troublesome throughout most of the rice producing regions of the United States and the world (Gealy et al. 2003, 2005; Bagavathiannan 2011; Norsworthy et al. 2013; Chauhan et al. 2017). Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv) interference can reduce rice yields up to 68% (Smith 1988), and junglerice (*Echinochloa colona* (L.) Link) can reduce yields up to 85% (Chauhan et al. 2017). Both species are strong competitors with rice because these 1) are C4 grasses, which are more efficient and adaptable to stress than C3 rice (Bagavathiannan 2014; Talbert and Burgos 2007); 2) mimic the rice crop making it near indistinguishable for hand-removal or spot spraying (Barrett 1983; Gould 1991); and 3) have the ability to emerge and flourish in flooded conditions (Chauhan et al. 2017). These attributes make *Echinochloa* species most difficult to manage in a rice field, or eradicate once introduced.

Herbicide-based weed control has been the standard practice in rice production in the US and worldwide. Junglerice and barnyardgrass are the most problematic species in Arkansas rice production and these species have evolved resistance to propanil, quinclorac, clomazone, imazethapyr, and fenoxaprop (Heap 2018). Resistance to a single herbicide mode of action

(MOA) and resistance to multiple MOAs have been confirmed among Arkansas junglerice and barnyardgrass populations. The resistance mechanisms are described in two general categories: a) target-site resistance (TSR), which refers to the alteration of an herbicide target, thereby reducing the herbicide binding and b) non-target-site resistance (NTSR), which refers to increased detoxification, reduced uptake/translocation of the herbicide, or other mechanisms outside of the herbicide target (Powles and Yu 2010; Jugulam and Godar 2013). Some of these resistance mechanisms may result in fitness costs due to the extra energy required to achieve certain NTSR mechanisms or due to reduced efficiency of mutant (resistant) target enzymes. Fitness costs have been associated with some ACCase (Menchari et al. 2008; Darmency et al. 2014), quinclorac resistance (Jugulam and Godar 2013), atrazine resistance (Holt et al. 1993), and some mutant ALS (Alocer-Ruthling et al. 1992). Herbicide resistance does not always result in observable fitness costs. Bagavathiannan et al. (2011) observed no fitness costs associated with propanil-resistant and clomazone-resistant barnyardgrass populations in Arkansas. However, many fitness cost studies of herbicide-resistant populations are constrained by the absence of a genetically similar reference population, which limits the conclusions that can be drawn (Menchari et al. 2008; Schaedler et al. 2015).

Fitness costs can be manifested in various ways such as reduction in plant height and seed number (Holt et al. 1993). The total effect of a resistance trait may be manifested in competitiveness and can be gauged in a competition experiment. Replacement series studies have been widely used to understand interference interaction between two species (Cousens 1991; Gealy et al. 2005). The varying ratios of each species in the replacement series allows for better understanding of a competition hierarchy (Hoffman and Buhler 2002). This approach had been used in studying the interference between rice and *Fimbristylis miliacea* (L.) Vahl

(Schaedler et al. 2015); different cultivars of rice and barnyardgrass (Gealy et. al 2003); herbicide-resistant and -sensitive ecotypes of the same species (Bagavathiannan et al. 2011); and between plants of the same species (Hoffman and Buhler 2002). Little is known about the effect of single- and/or multiple-herbicide resistance on competitive ability. With the increasing occurrence of multiple herbicide resistance in *Echinochloa* spp. (or other weed species), it is important to know if such traits could affect weed-competitive ability. This would inform us in adjusting crop management practices. Using highly resistant (R1) and resistant (R2) lines derived from the same field populations of single- and multiple-resistant ecotypes, studies were conducted to determine: 1) if single- or multiple-herbicide-resistant junglerice differ in competitive ability; 2) if biotypes with high resistance to a herbicide differ in competitive ability than biotypes with lesser level of resistance from the same population; ; and 3) if certain herbicide resistance traits are more likely to reduce weed-competitive ability.

Seed source. Junglerice accessions were collected from rice-producing counties in Arkansas between 2010 and 2016 according to the methodology outlined by Burgos (2015). Samples were collected from fields reported to crop consultants or University Extension personnel as having populations that survived at least one herbicide application. Panicles were bulked by site in the field and by plant type. Sample size ranged from panicles of a few plants (all that existed in a small patch) to about 200 g of seed (representing a large patch of one plant type). Samples were placed in paper bags and allowed to dry at room temperature. When possible, field history was obtained.

The field-collected samples were tested with common rice and soybean (*Glycine max* (L.) Merr) herbicides to determine their respective herbicide resistance profiles (data not shown). Square pots (10-cm by 10-cm) were filled 2.5cm from the top with LC1 potting mix (Sun Gro

Horticulture, Seba Beach, AB, Canada) and ten seeds were planted in each pot. A total of 10 pots were planted with seeds per pot of each accession and uniform seedlings were thinned at one-leaf stage to one seedling per pot. At two-leaf stage seedlings were sprayed with herbicides (Table 4.1) with the recommended adjuvant (0.25% non-ionic surfactant or 1% crop oil concentrate). Herbicides were applied in a spray chamber with a motorized boom fitted with two 8002 flat-fan nozzle tips (TeeJet Technologies, P.O. box 7900 Wheaton Il 60187) in a carrier volume of 187 L ha^{-1} at a speed of 1.6 kph and height of 75 cm above the spray target. Three weeks after treatment (WAT), the seedlings were evaluated for injury relative to the non-treated controls and the number of survivors counted (data not shown). Eight of these samples were selected based on their resistance profile, which included single- and multiple-resistant populations (Table 4.1). Accessions were verified as junglerice based on morphological traits (Tahir et al. 2016). Attempts were made to segregate susceptible (S) biotypes within each accession by spraying sub-lethal herbicide doses at three-four leaf stage. Ten seeds of each accession were planted into 10- x 10-cm pots. Ten pots were planted per herbicide per accession and seedlings were thinned to one plant per pot. The most uniform seedlings were selected. Seedlings were sprayed at three-four leaf stage with a 1x rate of propanil or quinclorac or ¼ x rate of cyhalofop, imazethapyr, or glufosinate. Applying low rates of herbicides was intended to select sensitive plants, which were expected to sustain injury, but survive and produce seed. Injury (stunting, chlorosis, necrosis, reduced tillering) was evaluated 2 WAT, relative to the control plants. Individuals showing more than 60% injury were classified as sensitive while those with less than 30% injury were classified as resistant. The selected S and R biotypes were then transplanted into 18- x 10-cm pots filled with the same volume of field soil (Captina Silt loam-fine-silty, siliceous, mesic Typic Fragiudults) from the Arkansas Agricultural Research and Extension Center Fayetteville,

Arkansas. Seeds of twice-selfed lines were used in succeeding experiments. Bioassays of 'S' and 'R' biotypes showed that the field-collected samples did not have true sensitive plants, although different levels of resistance was observed within the population. The selected biotypes with different resistance levels were kept separate and classified R1 (highly resistant) and R2 (resistant) (seeChapter 3).

Competition of rice with various herbicide-resistant *E. colona*. The experiment was conducted in a greenhouse at the Althiemer Laboratory without supplemental lighting, but was temperature-controlled (27-35°C). Pots, 20 cm in diameter and 20 cm tall, were filled with 4 kg of dried field soil (Captina Silt loam-fine-silty, siliceous, mesic Typic Fragiudults) collected from the Arkansas Agricultural Research and Experiment Center. The biotypes of eight accessions described above (Table 4.1) were planted with Roy J rice, which is a conventional inbred planted extensively in Arkansas. The experiment was setup in a randomized complete block design with benches being the blocking variable. The experiment was conducted twice; from July-August 2016 and from April -May 2017. Seeds were germinated on Petri plates and seedlings with 1-cm shoots were transplanted to the pots. Each pot had two plants. The treatments consisted of monocultures of R1 or R2 biotypes as well as Roy J rice and mixed plantings (1:1) of R1:R2, R1:rice, or R2:rice. Data collected included height (cm) from soil surface to the leaf tip on the tallest tiller and number of tillers every two weeks until termination of the experiment 6 weeks after planting. This time period falls within the critical weed-free period for rice, which ranges from 2 to 10 weeks after planting (Singh et al. 2014). At termination, aboveground biomass of each plant was harvested, oven-dried for one week at 30°C, and weighed.

Replacement Series Experiment: single- or multiple-resistance *E. colona* vs rice.

Experiments were conducted in a greenhouse at the Althiemer Laboratory, University of Arkansas, and Fayetteville, AR from August 2016 to May 2017 set at 12-hour days with temperature ranging from 32-37 °C. The plants were established in a raised-bed, 3.2 m x 6.4 m, built using 40 x 17.5-cm cinder blocks lined with 6 mm plastic and filled with field soil (Captina Silt loam-fine-silty, siliceous, mesic Typic Fragiudults) to a depth of 20 cm. The experiment had four replications organized in a randomized complete block with two temporal replications. Each run was conducted over a 6-week duration, which falls within the critical weed-free period of rice. The total plant density per plot was 38 plants m^{-2}. For rice monoculture, this was equivalent to the commercial planting recommendation of 32-64 plants m^{-2} for conventional rice on a silt loam soil in Arkansas (Hardke et al. 2016). The bed was flooded one week after transplanting and maintained at a flooded depth of 15 cm until 5 d before termination of the experiment. Each plot was 30 cm^2 with 12 plants spaced equidistantly. A weed-free barrier of 30 cm^2 separated each plot on all sides. Two junglerice accessions were used: R1 and R2 biotypes of Eco-45 (highly resistant to propanil and quinclorac with low-level resistance to cyhalofop and elevated tolerance to glufosinate) and Eco-76 (highly resistant to quinclorac). The rice variety was Roy J, as in the above experiment. Seedlings of junglerice and rice were raised in the greenhouse in 50 x 40-cm trays filled with LC1 potting mix (Sun Gro Horticulture, Seba Beach, AB, Canada). The seedlings were transplanted to the rice bed at 3-4 leaf stage. Junglerice and/or rice were planted at the following proportion s- 100:0 (rice monoculture) 75:25, 50:50, 25:75, and 0:100 (junglerice monoculture). For the R1 and R2 interference, 100:0 was R1 monoculture and 0:100 was R2 monoculture.

Data collected included height (cm) from soil surface to the leaf tip of the tallest tiller and number of tillers once every two weeks of the center two plants in each plot until termination. The experiment was terminated 6 weeks after planting and aboveground biomass of each plant was harvested, oven dried at 30°C for one week, and weighed.

Statistical Analysis

Data were analyzed using JMP Pro 13 (Statistical Analysis Systems Institute, SAS Circle P.O. Box 8000 Cary NC 25712-8000). An ANOVA was conducted for the 1:1 study on growth parameters and significant means separated with Fischer's protected LSD (α=0.05). A Dunnett's test (α=0.05) was performed to compare the biomass, height, and number of tillers per plant for the plant mixtures (25:75, 50:50, and 75:25) compared to the monoculture control (100:0). Dunnett's test compared plants of the same biotype across the mixtures and monocultures. A one-way ANOVA was conducted on all the monocultures for each respective biotype/species, for each of the growth parameters of interest, with significant means separated using Fisher's protected LSD (α=0.05). Runs were analysed as a fixed variable in both experiments due to differences in the growth of transplants between the runs. Indices described by Cousens and O'neill (1993) were calculated using the plant data to assess differences in competitive abilities.

The Relative Yield (RY) was calculated as follows:

1) $RY(A)= P(A_{mix}/A_{Mono})$

2) $RY(B)= (1-P)(B_{mix}/B_{Mono})$

P is the portion of the junglerice or rice present in the mixture and A and B are the yields of plants in mixtures (mix) or monoculture (mono).

The Relative Yield Total (RYT) was calculated using equation 3:

3) $RYT=RY(A)+RY(B)$

The actual RYT, RY(A), or RY(B) was then compared to the hypothesized value using a one-sample *t*-test (α=0.05). For example, in a 25:75 mixture the expected values are RYT = 1 and RY (A) = 0.25 and RY (B) = 0.75 if there is no interference; however, if the observed values are RYT = 0.8, RY(A) = 0.1, and RY(B) = 0.7, the coexisting species or biotypes may be interfering with one another. The *t*-test will determine if the observed values are different from expected values; ergo, if interference was significant and which species/biotype is more competitive.

The Competitive Ratio (CR) was calculated using equation 4:

4) $CR = [(1-P)/P][RY(A)/RY(B)]$

A one-sample *t*-test (α=0.05) was ran to determine if the CR deviates from the expected value of

The Relative Crowding Coefficient (RCC) was calculated using equation 5:

5) $RCC(A)=[(1-P)/P][RY(A)/(1-RY(A))]$

To compare each biotype in the mixtures (R1 vs R2, R1 vs Rice, R2 vs Rice) the difference between RCC(A) and RCC(B) was tested using a two-sample student's *t*-test (α=0.05).

The Aggressiveness index (AI) was calculated using equation 6:

6) $AI= (RY(A)/2P)-(RY(B)/[2(1-P)])$

A one sample *t*-test (α=0.05) was performed to determine if the AI deviated from the expected value of 1.

Results

Rice in 1:1 competition with HR *E. colona*. This experiment assessed the relative growth of R1 (highly resistant) and R2 (resistant) biotypes from various junglerice accessions with different resistance profiles in 1:1 competition with rice. Eco-76 was the tallest and Eco-225 was the shortest (the same height as Roy J rice) among the accessions in the first run of the experiment (Table 4.1). There were no differences between the R1 and R2 biotypes of any

accession in any of the growth variables evaluated. The number of tillers was not significantly different across all junglerice accessions and rice. Eco-76 and Eco-225 had the highest and lowest biomass, respectively, with no differences between biotypes. In the second run, the accessions did not differ in any of the growth variables evaluated.

The competitive indices CR, AI, RCC(A), and RCC(B) of each accession, biotype, or rice mixture did not differ from the reference values in either run (data not shown). Therefore, the accessions tested were equally competitive with rice, regardless of biotype. The data indicate that acquiring resistance to one or more herbicides (propanil, quinclorac, cyhalofop) did not change the competitiveness of junglerice in a 1:1 planting scheme.

Replacement Series Experiment: single- or multiple-resistant *E. colona* vs. rice. Monocultures of the R1 and R2 biotypes of Eco-45 or Eco-76 did not differ in biomass, height, or tiller number. Eco-45 had significantly greater biomass (17 g plant^{-1}) than Eco-76 (14 g plant^{-1}) and rice (9 g plant^{-1}) (Table 4.2). Eco-45 and Eco-76 were similar in height and biomass in the1:1 study, but in the replacement study Eco-45 had more tillers than Eco-76 and rice, which contributed to its high biomass production. Both monocultures of Eco-45 and Eco-76, regardless of biotype, had greater biomass than the rice monoculture. The heights of Eco-76 and Eco-45 were similar (140-146 cm) and both were taller than the rice (Table 4.2).

The shape of the RY curves of each competing species or biotypes is indicative of the degree of interference that is occurring. If the line is concaved, then competitor A is negatively affected by competitor B; if it is convex then competitor A is superior to competitor B (Harper1977; Joliffe 2000). In every junglerice/rice mixture, the RY line for biomass of junglerice, regardless of the accession or biotype, was convex while that of rice was concave (Figure 4.1 and 4.2), indicating that junglerice was more competitive than the rice. When

junglerice, the RY values of biomass of Eco-76 were 0.15-0.24 higher than the no-interference reference values (Table 4.4); those of Eco-45 were 0.1-0.24 higher than the reference (Table 4.3). The RY lines for Eco-76R2 and Eco-76R1 in competition with rice were more convex than those of Eco-45R2 and Eco-45R1 (Figure 4.1-4.2). Together, this indicates that Eco-76 is more competitive than Eco-45 with Roy J rice. Hoffman and Buhler (2002) proposed that if the competitive ratio (CR) is >1, the RCC (A) is > relative RCC (B), and AI is > 0 then species A is more competitive than species B. This criteria classify Eco-76 as more competitive than rice. When grown together, Eco-76R1 and Eco-76R2 were equally competitive with each other (Table 4.5). Having extreme high resistance to quinclorac did not compromise the competitive ability of this accession, compared to a biotype with relatively lower level of resistance. Collectively, this indicates that resistance to quinclorac alone did not compromise the competitive ability of junglerice. The competitive indices of Eco-45R2 and Eco-45R1 grown with rice did not differ from the reference values (Table 4.6). Therefore, R1 and R2 biotypes of Eco-45 were also equally competitive with rice. Multiple resistance to propanil and quinclorac with tolerance to glufosinate and cyhalofop did not reduce the competitive ability of Eco-45. The biomass per plot of Eco-76 was greater in mixtures with rice compared to that of the Eco-76 monocultures (Table 4.7). Interspecies competition with rice was less intense than intraspecies competition among junglerice plants. Hence, junglerice grew bigger when some rice were in the mixture compared to 100% junglerice.

Discussion

The absence of differences in competition indices across accessions in the 1:1 competition study, despite accession differences in plant sizes, indicates that the number of plants for the pot size was less than optimum, resulting in less intense competition between the

two plants. This was not expected since both junglerice and rice produce tillers and expand their areas of influence quickly. Low plant densities allow avoidance of competition, which is a common critique of replacement series studies (Joliffe 2000). To verify this, a planting density experiment needs to be conducted to determine the carrying capacity for this pot size then this experiment should be repeated using that population density.

In the replacement study, Eco-76 was significantly more competitive with rice than Eco-45. The increased aggressiveness of Eco-76 or the lower competitive ability of Eco-45 cannot be contributed to the resistance traits of these accessions because the biotypes of either accession did not differ in aggressiveness or competitive ability. Differences between Eco-76 and Eco-45 are due to species population diversity or ecotype differentiation apart from the influence of herbicide selection pressure (Darmency et al. 2014). Further investigation into the mechanisms endowing resistance in these accessions could provide insight about the lack of effect on competitive ability of both accessions. Although both biotypes from these accessions were equally competitive, there are a few cases in the literature where R plants were less fit than their S counterparts. Some resistance mechanisms cause fitness penalties while others do not. It was found that the most common occurring target site mutation in ACCase-herbicide-resistant blackgrass (*Alopecurus myosuroides* (L)) (Leu_{1781}) did not cause fitness penalties; however, one of the least common mutations at Gly_{2078} was associated with decreased biomass (Menchari et al. 2006; 2008). The rarity of a particular target site mutation is a consequence of its negative effect on the plant because weak individuals are eliminated from the population. Conducting a replacement series experiment between Eco-45 and Eco-76 would demonstrate if Eco-76 is a superior competitor not only to rice but also to other junglerice populations. These experiments were not carried to maturity, which prevented the assessment of competition impact on fecundity

and prediction of future population size. These experiments were designed to capture competition during the critical weed-free period for rice (Howell 1990; Singh et al. 2014).

Echinochloa spp. have high propensity to evolve resistance to herbicides, second only to rigid ryegrass (*Lolium rigidum*) in terms of global resistance cases (Heap 2017). One reason for the widespread evolution of resistance in *Echinochloa* spp. is that rice is produced on such a large area across wide-ranging agroclimatic environments worldwide and these species are among the most common rice weeds (Chauhan et al. 2017). Junglerice can establish and thrive in direct- seeded rice which is becoming more popular due to water and labor scarcity, but also thrives in flooded rice culture (Chauhan et al. 2017). In dry-seeded rice production systems chemical control is the main weed control method, so the junglerice/barnyardgrass complex is exposed to herbicide selection pressure more often than strictly aquatic or strictly terrestrial species. Another major factor contributing to resistance evolution is the high genetic variability and high ploidy of these species (Tahir and Burgos 2016). Junglerice populations in Arkansas have large phenotypic variability in growth habits and maturity parameters (Tahir and Burgos 2016). Barnyardgrass is a hexaploid and other members of *Echinochloa spp*. are diploid (Ye C et al. 2014). Species with high ploidy can hybridize (Snustad and Simmons 2009), and they can produce a highly diverse offspring. Both barnyardgrass and junglerice are characterized as self-pollinators, but studies have found sufficient rates of cross pollination to occur between species to allow for gene exchange (Tabacchi et al. 2006). This hybridization can contribute to the further spread of resistance mutations across these species within the state. Some junglerice ecotypes within Arkansas are difficult to phenotypically distinguish from barnyardgrass (Tahir and Burgos 2016). It is possible that, due to this large genetic and phenotypic variability, plants

carrying resistance mechanisms with fitness penalties are eliminated from the population by dominance of resistant plants that are more fit.

Conclusions

Junglerice populations in Arkansas vary significantly in plant size and growth habit. Differences in height, biomass, or tillers are not associated with resistance traits, but with ecotype. All junglerice accessions (regardless of biotype) are more competitive than Roy J rice. The R1 and R2 biotypes of any accession have the same competitive ability. Based on the replacement series study, Eco-76 is a stronger competitor with rice than Eco-45. Intraspecific competition among junglerice plants is stronger than interspecific competition of junglerice with rice. Multiple resistance to propanil, quinclorac and low tolerance to glufosinate and cyhalofop have no effect on the competitive ability of junglerice. The competitiveness of junglerice is related to plant size and morphology, but not to resistance traits.

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Tables

Table 4.1. Growth parameters of *E. colona* and rice grown in monocultures in the 1:1 competition experiment.

Accession-Biotype[a]	Profile[b]	Run	Plant height[c] (cm)	Tillers [c] (#)	Biomass[c] (g)
Rice-Roy J		1	70 ghi	7	53 efg
Eco-26S1		1	83 efgh	8	55 defg
Eco-26S2		1	84 efg	7	54 efg
Eco-35R1	P,Q	1	111 ab	7	62 ab
Eco-35R2		1	109 bc	7	62 ab
Eco-45R1	P,Q,C	1	108 bcd	10	63 ab
Eco-45R2		1	109 bc	11	60 abcde
Eco-76R1	Q	1	125 a	7	62 ab
Eco-76R2		1	118 ab	7	64 a
Eco-101R1	P,Q,I	1	86 ef	6	58 abcdef
Eco-101R2		1	95 de	6	57 bcdef
Eco-208R1	Q,C	1	96 cde	8	61 abc
Eco-208R2		1	95 de	8	61 abcd
Eco-225R1	P,I	1	70 hi	6	51 g
Eco-225R2		1	69 i	7	53 fg
Eco-245R1	Q,C	1	83 efgh	8	58 abcdef
Eco-245R2		1	79 fghi	8	55 cdefg
P-value			*<0.0001*	*0.1*	*0.0016*
Rice-Roy J		2	44	2	27
Eco-26S1		2	37	3	27
Eco-26S2		2	34	3	28
Eco-35R1	P,Q	2	29	3	27
Eco-35R2		2	33	3	27
Eco-45R1	P,Q,C,G	2	40	5	29
Eco-45R2		2	36	5	28
Eco-76R1	Q	2	34	1	28
Eco-76R2		2	41	2	27
Eco-101R1	P,Q,I	2	36	3	28
Eco-101R2		2	34	4	27
Eco-208R1	Q,C	2	32	3	27
Eco-208R2		2	29	4	28
Eco-225R1	P,I	2	41	3	28
Eco-225R2		2	38	4	27
Eco-245R1	Q,C	2	30	3	27
Eco-245R2		2	35	3	27
P-value			*0.22*	*0.84*	*0.69*

[a] Accessions with single- and multiple herbicide resistance. Highly resistant (R1) resistant (R2) Sensitive (S1) and highly sensitive (S2) biotypes are included as well as Roy J rice (Rice).
[b] Resistance profile ; P= propanil, Q= quinclorac, C=cyhalofop, I=imazethapyr.
[c]In a column, different letters indicate significant differences (α=0.05).

Table 4.2. Growth response parameters of *E. colona* and rice grown in monocultures for the replacement series experiment at a density of 38 plants per m^2.

Accession	Biotype[a]	Plant Height (cm)[b]	Tillers $plant^{-1}$(#)	Biomass (g)
ECO-45	R2	142.7 a	6 a	17.8 a
	R1	140.3 a	6 a	17.8 a
ECO-76	R2	146.5 a	4 b	14.7 b
	R1	146.5 a	4 b	14.4 b
Rice	-	91.3 b	4 b	9.9 c
P-value		*<0.0001*	*0.005*	*<0.0001*

[a] Accessions with single- and multiple herbicide resistance. Highly resistant (R1) resistant (R2).
[b] Means followed by the same letter within a column are not significantly different using Fisher's Protected LSD (α=0.05).

Table 4.3. Relative yield (RY) and relative yield total (RYT) of tillers and dry biomass (g) of Eco-45 for each plant combination.

Proportions[a]	Tillers[b]			Biomass[c]		
	75:25	50:50	25:75	75:25	50:50	25:75
Eco-45R2 vs.Rice						
RY (R2)	0.074 (-0.01)	0.49 (-0.01)	0.20 (-0.05)	0.79 (+0.04)	0.6 (+0.1)*	0.4 (+0.21)*
RY (Rice)	0.1 (-0.15)*	0.23 (-0.27)*	0.75 (0)	0.54 (+0.29)*	0.52 (+0.02)	0.65 (-0.1)*
RYT	0.73 (+0.27)*	0.77 (-0.23)*	0.95 (-0.05)	1.33 (+0.33)*	1.12 (+0.12)	1.1 (+0.1)*
Eco-45R1 vs.Rice						
RY (R1)	0.71 (-0.04)	0.54 (+0.04)	0.3 (+0.05)*	0.82 (+0.07)	0.69 (+0.19)*	0.49 (+0.24)*
RY (Rice)	0.13 (-0.12)*	0.34 (-0.16)	0.43 (-0.32)*	0.56 (+0.31)*	0.62 (+0.12)*	0.62 (-0.13)*
RYT	0.74 (-0.26)*	0.88 (-0.12)	0.72 (-0.28)*	1.26 (+0.26)*	1.33 (+0.33)*	1.11(+0.11)*
Eco-45R1 vs.R2						
RY (R2)	0.73 (-0.02)	0.48 (-0.02)	0.25 (0)	0.21 (-0.04)*	0.57 (+0.07)*	0.42 (+.17)*
RY (R1)	0.25 (0)	0.50 (0)	0.75 (0)	0.42 (+0.17)*	0.56 (+0.06)	0.81 (+0.06)
RYT	0.97 (-0.03)	0.96 (-0.04)	1.0 (0)	1.13 (+0.13)*	1.14 (0.14)*	1.23 (0.23)*

[a]Mixtures of highly resistant (R1)and resistant (R2) Eco-45 and rice

[b] RY and RYT of tillers per plant from mixtures; values in parenthesis are differences between observed values and expected values (*Ho*)

[c] RY and RYT of biomass per plot from mixtures; values in parenthesis are differences between observed values and expected values (*Ho*)

*indicates significant differences in observed values and *Ho* using a one sample *t*-test (α=0.05).

Table 4.4. Relative yield (RY) and relative yield total (RYT) of tillers (#) and dry biomass (g) of Eco-76 and rice for each combination

Proportions[a]	Tillers[b]			Biomass[c]		
	75:25	50:50	25:75	75:25	50:50	25:75
Eco-76R2 vs.Rice						
RY (R2)	1.03 (+0.28)	0.53 (+0.03)	0.33 (+0.08)	1.0 (+0.25) [d]	0.71 (+0.21) [d]	0.46 (+0.21) [d]
RY (Rice)	0.21 (-0.04)	0.42 (-0.08)	0.61 (-0.14)	0.55 (+0.3) [d]	0.53 (+0.03)	0.67 (-0.08) [d]
RYT	1.06 (+0.06)	1.09 (+0.06)	0.94 (-0.06)	1.2 (+0.2)	1.25 (0.25) [d]	1.22 (+0.22) [d]
Eco-76R1 vs.Rice						
RY (R1)	0.78 (+0.03)	0.53 (+0.03)	0.32 (+0.07)	0.91 (+0.15)	0.74 (+0.24) [d]	0.4 (+0.15)
RY (Rice)	0.15 (-0.1)[d]	0.4 (-0.1)	0.54 (-0.21)[d]	0.58 (+0.33)[d]	0.57 (+0.07) [d]	0.63 (-0.12) [d]
RYT	0.92 (-0.08)	0.92 (-0.08)	0.86 (-0.14)	1.36 (+0.36) [d]	1.28 (+0.28) [d]	1.1 (+0.1)
Eco-76R1 vs. R2						
RY (R2)	1.07 (+0.32)	0.65 (+0.15)	0.32 (+0.07)	0.84 (+0.09)	0.52 (+0.02)	0.35 (+0.12) [d]
RY (R1)	0.23 (-0.02)	0.4 (-0.1) [d]	0.9 (+0.15)	0.59 (+0.34) [d]	0.74 (+0.24) [d]	0.94 (+0.19)
RYT	1.36 (+0.36)	0.86 (-0.14)	1.23 (+0.23)	1.35 (+0.35) [d]	1.12 (+0.12)	1.18 (+0.18) [d]

[a]Mixtures of highly resistant (R1)and resistant (R2) Eco-76 and rice

[b] RY and RYT of tillers per plant from mixtures; values in parenthesis are differences between observed values and expected values (*Ho*)

[c] RY and RYT of biomass per plot from mixtures; values in parenthesis are differences between observed values and expected values (*Ho*)

[d] indicates significant differences in observed values and *Ho* using a one sample *t*-test (α=0.05).

Table 4.5 Indices for replacement series with *E. colona* and rice combinations.

Mixture[a]	Indices-Biomass[b]						
	CR	P value[c]	RCC(A)	RCC(B)	P value[d]	AI	P value[e]
Eco-45R2 vs.Rice	0.85	0.1	rice-1.32	S-1.6	0.41	-0.08	0.1
Eco-45R1 vs.Rice	0.92	0.38	rice-1.89	R-1.99	0.78	-0.07	0.3
Eco-45R1 vs.R2	0.98	0.75	R-1.45	S-1.49	0.89	-0.01	0.64
Eco-76R2 vs.Rice	0.74*	0.02*	rice-1.5	S-2.3	0.12	-0.17*	0.03*
Eco-76R1 vs.Rice	0.81*	0.03*	rice-1.3	R-2.4	0.27	-0.15*	0.04*
Eco-76R1 vs.R2	1.4	0.09	R-1.5	S-1.1	0.26	0.21	0.1

[a] Mixtures of R and S *E. colona* and Rice.

[b] Indices were calculated from the biomass of mixtures with equal portions i.e. 50:50; CR= competitive ratio; RCC(A) and RCC(B)=relative crowding coefficient of the R, S, or rice; AI=aggressiveness index.

[c] P values of CR from one sample *t*-test (α=0.05) to determine if deviation of the CR from 1 was significant.

[d] P values of RCC(A) and RCC(B) comparisons with a Student's *t*-test (α=0.05).

[e] P values of AI from one sample *t*-test (α=0.05) to determine if deviation of the AI from 0 was significant.

Table 4.6. Growth response parameters of *E. colona* (Eco-45) biotypes grown in replacement series.

Proportion[a]	Plant height [b] (cm)		Tillers [b] (#)		Biomass [b] (g)	
Eco-45R2 vs.Rice	R2	Rice	R2	Rice	R2	Rice
Check (100)	142.7	91.7	6	4	17.8	9.9
75:25	134.2	79.0	7	5	18.6	20.3*
50:50	135.1	68.1*	8	2	21.8	11.4*
25:75	141.2	75.0	9	3	33.1*	8.5*
P value[c]	*0.99*	*0.04**	*0.08*	*0.88*	*<0.0001**	*0.02**
Eco-45R1 vs.Rice	R1	Rice	R1	Rice	R1	Rice
Check (100)	140.3	91.3	7	4	17.8	9.9
75:25	136.2	85.5	5	3*	19.2	20.7*
50:50	141.6	79.1	4	2*	24.2	12.3*
25:75	142.8	84.5	8	2*	35.5*	8.1*
P value[c]	*0.99*	*0.77*	*0.3*	*0.002**	*0.003**	*0.003**
Eco-45R1 vs.R2	R2	R1	R2	R1	R2	R1
Check (100)	142.7	140.7	6	6	17.8	17.8
75:25	137.6	139.3	5	5	16.9	18.6
50:50	135.5	140.6	5	6	20.8	19.6
25:75	143.6	140.2	6	5	29.9*	29.0*
P value[c]	*0.99*	*0.99*	*0.98*	*0.96*	*0.0002**	*0.002**

[a] Biotypes and accessions in respective proportions and ratios.

[b] Within each column, the evaluated mixtures for each biotype/species are compared to the monoculture control using Dunnett's test (α=0.05).

[c]P values are from Dunnett's test with * indicating significant p values and differences in mixtures vs. monocultures.

Table 4.7. Growth response parameters of *E. colona* (Eco-76) biotypes grown in replacement series.

Proportion [a]	Plant height [b] (cm)		Tillers [b] (#)		Biomass [b] (g)	
Eco-76R2 vs.Rice	R2	Rice	R2	Rice	R2	Rice
Check (100)	142.5	86.2	3	2	14.9	9.1
75:25	162.6*	76.3	5	2	16.9	19.9*
50:50	157.2	74.7	4	2	21.7*	9.5
25:75	160.4*	82.2	4	2	32.6*	7.9
P value[c]	*0.041**	*0.63*	*0.46*	*0.28*	*0.006**	*<.0001*
Eco-76R1 vs.Rice	R1	Rice	R1	Rice	R1	Rice
Check (100)	147.3	90	4	2	14.5	9
75:25	150.4	64.8	4	1	17.6	19.2*
50:50	151	81	4	2	21.7*	9
25:75	146	69.7	5	1	32.1*	7*
P value[c]	*0.99*	*0.23*	*0.57*	*0.53*	*0.003**	*0.007**
Eco-76R1 vs.R2	R2	R1	R2	R1	R2	R1
Check (100)	134.9	146.7	3	4	14.9	14.5
75:25	143.5	137.4	4	4	15.7	23.9*
50:50	148.6	138	4	3	15.7	16.1
25:75	142.4	147.6	4	4	25.3*	12.8
P value[c]	*0.47*	*0.65*	*0.34*	*0.58*	*<0.0001*	*<0.0001*

[a] Biotypes and accessions in respective proportions and ratios.

[b] Within each column, the evaluated mixtures for each biotype/species are compared to the monoculture control using Dunnett's test (α=0.05).

[c] P values are from Dunnett's test with * indicating significant p values and differences in mixtures vs. monocultures.

Figures

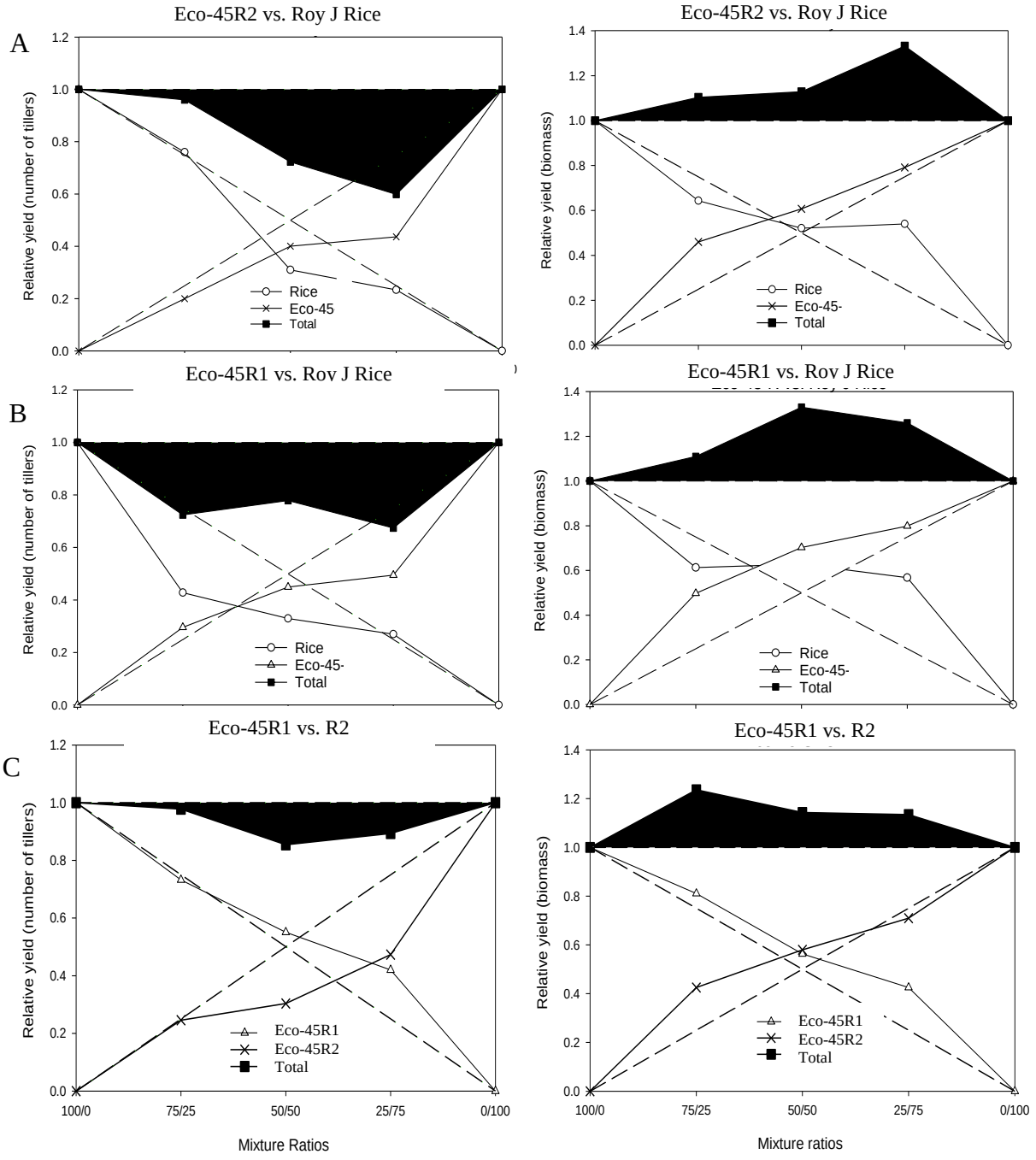

Figure 4.1 (a-c) Relative yield totals (RYT) and Relative yields (RY) for mixture ratios calculated for dry biomass and tillers for (A) Eco-45-R2 vs. rice (B) Eco-45-R1 vs. rice (C) Eco-45R1 vs R2. The dashed lines represent the hypothetical lines of each species if they are equally competitive according to (Jolliffe 2000).

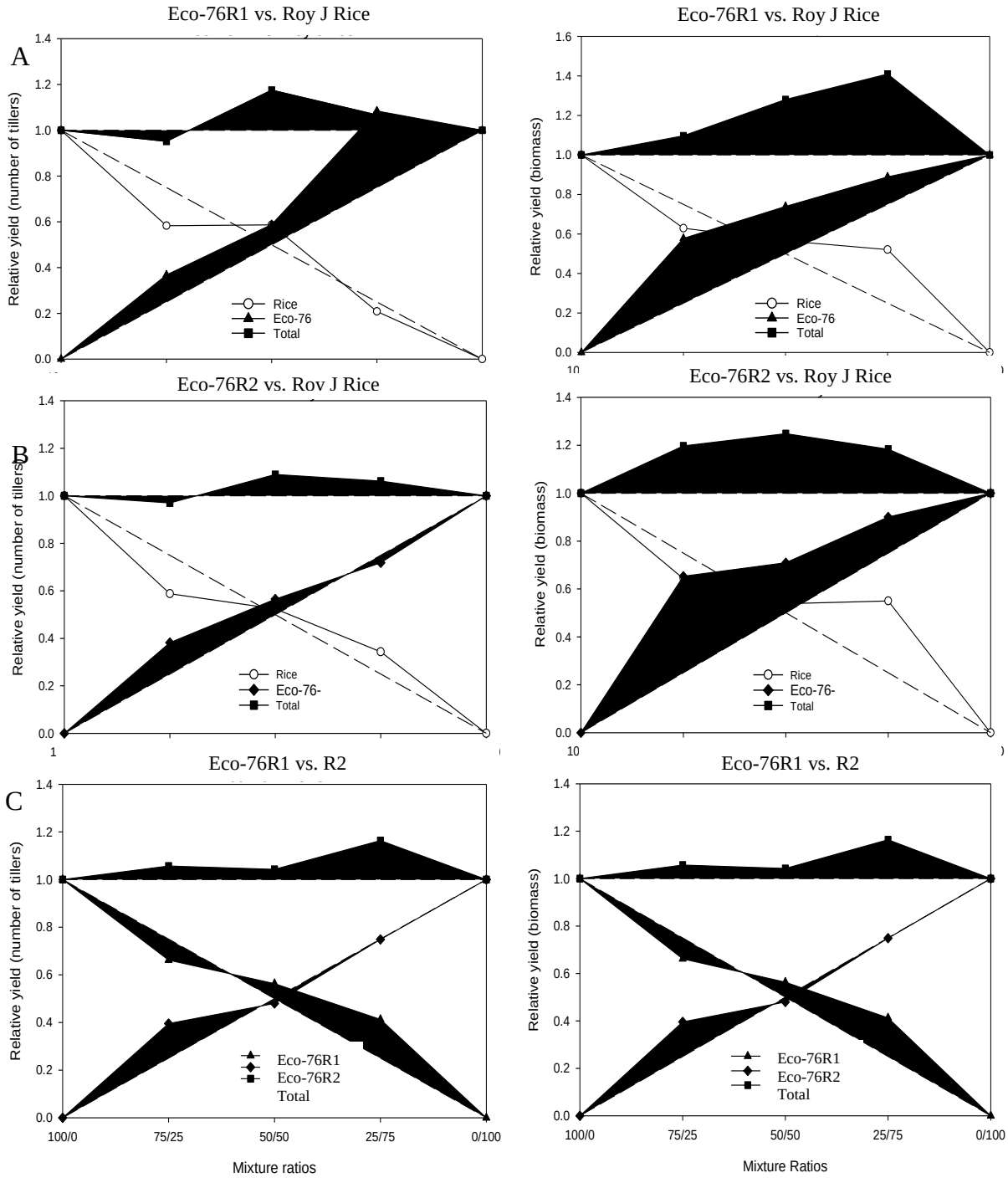

Figure 4.2 (a-c) Relative yield totals (RYT) and Relative yields (RY) for mixture ratios calculated for dry biomass and tillers for (A) Eco-76-R1 vs. rice (B) Eco-76-R2 vs. rice and (C) Eco-76-R1 vs R2. The dashed lines represent the hypothetical lines of each species if they are equally competitive according to (Jolliffe 2000).

Appendix

Appendix A**.** Growth parameters of highly resistant and resistant *E. colona* grown with rice for the 1:1 competition experiment, Run 1.

Accession-Biotype[a]	Profile[b]	Plant height (cm)			Tillers (#)			Biomass (g)		
		R1	R2	Rice	R1	R2	Rice	R1	R2	Rice
Eco-26	SS	60	65	44	4	5	2	63	56	49
Eco-35	P,Q	78	74	50	6	6	2	67	70	54
Eco-45	P,Q	66	66	55	7	7	3	61	63	48
Eco-76	Q	82	81	54	6	5	2	64	63	47
Eco-101	P,Q,I	64	61	47	6	6	2	51	57	49
Eco-208	Q	69	69	50	7	7	2	62	61	53
Eco-225	P,I	55	53	48	6	5	2	50	51	48
Eco-245	Q	58	56	43	6	6	2	52	52	50
P-value			*ns*			*ns*			*ns*	

[a] Accessions with single- and multiple herbicide resistance. Highly resistant (R1) and resistant (R2) biotypes are included as well as Roy J rice (Rice).

[b] Resistance profile ; SS = susceptible to all herbicides, P= propanil, Q= quinclorac, C=cyhalofop, I=imazethapyr

Appendix B. Indices for replacement series with *E. colona* and rice combinations in 1:1 competition study.

Proportions[a]	Indices-Biomass[b]						
	CR	P value[c]	RCC(A)	RCC(B)	P value[d]	AI	P value[e]
Eco-245R2 vs. Rice	0.97	0.74	rice-0.04	R2-0.03	0.34	-0.02	0.70
Eco-245R1 vs. Rice	1.02	0.69	rice-0.04	R1-0.05	0.51	0.01	0.64
Eco-245R1 vs. R2	1.13	0.12	R1-0.03	R2-0.03	0.27	0.06	0.12
Eco-225R2 vs. Rice	0.93	0.53	rice-0.05	R2-0.01	0.68	-0.04	0.52
Eco-225R1 vs. Rice	0.87	0.19	rice-0.07	R1-0.01	0.87	-0.06	0.14
Eco-225R1 vs. R2	0.87	0.35	R1-0.07	R2-0.01	0.78	-0.06	0.33
Eco-208R2 vs. Rice	0.97	0.81	rice-0.01	R2-0.00	0.94	-0.02	0.76
Eco-208R1 vs. Rice	0.99	0.70	rice-0.00	R1-0.00	0.91	-0.01	0.68
Eco-208R1 vs. R2	0.97	0.64	R1-0.01	R2-0.00	0.94	-0.02	0.67
Eco-101R2 vs. Rice	0.96	0.64	rice-0.04	R2-0.02	0.52	-0.02	0.61
Eco-101R1 vs. Rice	0.94	0.38	rice-0.08	R1-0.05	0.36	0.03	0.41
Eco-101R1 vs. R2	1.11	0.26	R1-0.02	R2-0.03	0.10	0.05	0.26
Eco-76R2 vs. Rice	0.88	0.22	rice-0.07	R2-0.01	0.70	-0.06	0.19
Eco-76R1 vs. Rice	1.01	0.95	rice-0.01	R1-0.01	0.92	-0.01	0.87
Eco-76R1 vs. R2	0.99	0.61	R1-0.04	R2-0.03	0.42	-0.01	0.65
Eco-45R2 vs. Rice	0.85	0.15	rice-0.05	R2-0.03	0.42	-0.08	0.17
Eco-45R1 vs. Rice	0.86	0.18	rice-0.09	R1-0.02	0.45	-0.07	0.20
Eco-45R1 vs. R2	1.00	0.76	R1-0.00	R2-0.00	0.92	0.0	0.73
Eco-35R2 vs. Rice	0.88	0.21	rice-0.00	R2-0.07	0.18	-0.07	0.23
Eco-35R1 vs. Rice	0.92	0.30	rice-0.01	R1-0.04	0.33	-0.04	0.10
Eco-35R1 vs. R2	1.01	0.91	R1-0.00	R2-0.00	0.92	0.00	0.98
Eco-26S2 vs. Rice	0.87	0.29	rice-0.05	S2-0.02	0.12	-0.07	0.29
Eco-26S1 vs. Rice	0.95	0.80	rice-0.06	S1-0.06	0.10	-0.03	0.73
Eco-26S1 vs. S2	0.97	0.83	S1-0.04	S2-0.08	0.30	-0.02	0.84

[a] Mixtures of highly resistant (R1) and resistant (R2) *E. colona* and Rice.

[b] Indices were calculated from the biomass of mixtures with equal portions i.e. 1:1; CR= competitive ratio; RCC(A) and RCC(B)=relative crowding coefficient of the R, S, or rice; AI=aggressiveness index.

[c] P values of CR from one sample *t*-test (α=0.05) to determine if deviation of the CR from 1 was significant.

[d] P values of RCC(A) and RCC(B) comparisons with a Student's *t*-test (α=0.05).

[e] P values of AI from one sample *t*-test (α=0.05) to determine if deviation of the AI from 0 was significant.

Chapter 5: Seed germination behavior of *Echinochloa colona* populations with different herbicide resistance profiles under temperature stress

Abstract

The evolution of multiple herbicide resistance in *Echinochloa spp.* may impact its fitness as some herbicide resistance traits are associated with fitness costs. Fitness costs can be manifested as reduced germination capacity, tolerance to abiotic stresses, or germination rates. The objectives of this research were to evaluate: 1) the effect of herbicide resistance traits on germination parameters of junglerice (*Echinochloa colona* (L.) Link) and 2) how sub-optimum (15-20°C) and above-optimum (40°C) temperatures affect germination parameters of highly resistant (R1) and resistant (R2) *Echinochloa colona* with the same genetic background. Eight accessions representing different resistance profiles were tested. One accession was highly resistant to two herbicides (propanil and quinclorac), with low-level resistance to cyhalofop and elevated tolerance to glufosinate. At 15°C, R1 plants from four accessions had reduced germination capacity (GC) compared to their R2 counterparts. Eco-76R1 (high resistance to quinclorac) had higher GC than the Eco-76R2. At optimum temperature (30°C) and above-optimum (40°C), the germination of biotypes were generally similar with two exceptions. Eco-35R1 (resistant to propanil and quinclorac) germinated less than Eco-35R2. Eco-26S1 maintained its higher GC at 30°C compared to its S2 counterpart (both susceptible to cyhalofop). Overall, the largest differentiation in germination (1-98% GC) occurred at the lowest temperature tested (15°C). The R1 biotypes of Eco-35, 45, 101, 225, and 245 had lower GC at 15°C than the R2 biotypes. The R1 biotypes of Eco-76, and 208 all had higher GC than the R2 counterparts at 15°C. Both biotypes from all accessions germinated well (91-99%) at 40°C.

Introduction

Weeds have been the primary pest problem for crop producers since the beginning of crop cultivation (Grime 1977). The earliest weed control method involved removal of weeds by bare hands or with hand-held tools. This manual removal selected for weed species that looked and behaved similarly to the crop (Barrett 1983). This was observed with barnyardgrass (*Echinochloa crus-galli* (L) Beauv) in rice production. Barnyardgrass and other weedy *Echinochloa* species such as junglerice (*Echinochloa colona* (L) Link), early watergrass (*Echinochloa oryzicola* Vasing), and late watergrass (*Echinochloa phyllopogon* (Stapf) Koso-Pol) can withstand flooding, and mimic rice in growth habit. This mimicry phenomenon has given rise to ecotypes that could only be distinguished from rice based on the absence of a ligule in barnyardgrass (Barrett 1983, Gould 1991). Barnyardgrass can compete strongly with rice causing up to 68% yield loss (Smith 1988). Within the United States mid-south rice production area, the barnyardgrass/junglerice complex is the number one most troublesome weed due to the reasons mentioned above and the increasing evolution of herbicide resistance (Bagavathiannan et al. 2011; Malik et al. 2010; Norsworthy et al. 2013; Talbert and Burgos 2007).

Intense herbicide use has put yet another strong selection pressure on weeds. Since the commercialization and extensive use of propanil for rice weed control in 1959, it took almost 30 years before resistant barnyardgrass was detected in Arkansas, USA in 1990 (Talbert and Burgos 2007). This was followed by a succession of resistance evolution to other major herbicides used to control the barnyardgrass/junglerice complex including quinclorac, clomazone, and imazethapyr, which occurred in less than 10-year intervals (Heap 2017; Malik et al. 2010; Talbert and Burgos 2007). On a worldwide basis, barnyardgrass and junglerice has

evolved resistance to 10 sites of action, second only to rigid ryegrass (*Lolium rigidum* (Gaud)) (12 sites of action) (Heap 2017).

Resistance can be due to mutation(s) in the herbicide target site (TSR) or non-target site resistance (NTSR) mechanisms such as enhanced detoxification or reduced translocation of the herbicide in the plant. Some of these modifications, alone or in combination, can have pleiotropic effects, making the plant less fit (Panozzo et al. 2013; Sibony and Rubin 2002; Vila-Aiub 2009). Fitness costs associated with herbicide resistance was first documented involving atrazine due to a mutation in the D1 protein where atrazine binds, causing reduced photosynthetic efficiency, biomass production, and fecundity (Holt et al. 1993). Certain mutations in the acetolactate synthase (ALS) enzyme such as PRO_{197} result in reduced vigor (Yu and Powles 2009).

Fitness costs are often associated with a reduction in the number of offspring, but it is more appropriate to define fitness cost as any reduction or modification in physical or physiological processes (Vila-Aiub et al. 2015). These modifications could later result in reduced seed production or reduced seed viability under optimal or suboptimal conditions. We hypothesize that certain combinations of multiple resistance profiles can have negative effects on these physiological processes such as germination. This study was performed to understand the impact of different herbicide resistance profiles on the germination behavior of junglerice under low and high temperature stress. Potential differences in germination parameters could provide insight into how rapidly highly resistant (R1) or resistant (R2) populations germinate so that early herbicide applications can be timed to target the largest cohort of seedlings, thereby reducing in-season infestation. Understanding weed population differences in germination

behaviour also informs us on the benefit of earlier planting dates using cold-tolerant rice varieties as a component of an integrated weed management program.

Materials and Methods

Seed source. Junglerice accessions were collected in Arkansas from 2010 through 2014 and bioassayed for resistance to various herbicides. Eight of these accessions were selected based on their resistance profile (Table 5.1). Highly resistant (R1) and resistant (R2) biotypes were selected from each accession, selfed, and used in various experiments to assess potential fitness cost of the resistance trait. Seeds were stored at room temperature for two months prior to use.

Experimental Set-up. The germination experiments were conducted in incubation chambers (Diurna Growth 115V, VWR) at the Althiemer Laboratory, University of Arkansas, Fayetteville, AR from August 2016 through January 2017. Seeds of both biotypes were placed in Petri plates (50 per plate), lined with paper towel, and moistened with 20 ml of deionized water. The plates were incubated at 15, 20, 30, and 40°C with four replications per treatment. The optimum temperature was 30°C (Chauhan and Johnson 2009; Tahir and Burgos 2016). The incubation chamber was set to a 12-h photoperiod with constant day/night temperatures. Germinated seeds were counted every Monday, Wednesday, and Friday for four weeks (26 d). Seeds with visible radicle or shoot penetrating the seed coat were considered germinated. Germinated seeds were discarded. After 26 days, the viability of remaining seeds was determined using the tetrazolium assay (Peters and Lanham 2005). The experiment was repeated.

Statistical Analyses

The experiment was set-up in a split-plot design with the main plot being temperature and subplot being a factorial arrangement of accession and biotype. Data were subjected to ANOVA in JMP PRO 13 (Statistical Analysis Systems Institute, SAS Circle P.O. Box 8000 Cary NC

25712-8000). There was no significant difference between runs; thus, the data were pooled across runs. Regression analysis was done to estimate the time required for each accession to reach 50% germination (T_{50}) (Cousens 1988). Germination (%) was fitted to a three-parameter sigmoid model (Chauhan and Johnson 2009) using SigmaPlot v. 13 (Systat Software Inc):

$$G = G_{max} / [1+e(-(x-x_{50}) / G_{rate})]$$

where G is the cumulative germination (%) on day x, G_{max} is the maximum germination (%), x_{50} is the T_{50}, and G_{rate} is the slope. Accessions that did not fit this model were fitted with an appropriate model to estimate T_{50}.

Results and Discussion

Germination capacity was significantly affected by temperature, accession, and biotype ($P<0.0001$) (Table 5.2). Junglerice populations in Arkansas exhibit different germination capacities under optimal temperature, 30°C (Tahir et al. 2016). The extent of population differentiation in GC at low temperatures is not known. This information is important because crops are planted as early as producers can, in the spring, when the soil is still cool.

An interaction effect of accession x biotype or temperature x biotype on GC indicates that the resistance trait might have affected seed dormancy or seed germination response to temperature. At the lowest temperature (15°C), significant differences were observed between R1 and R2 biotypes of all accessions except Eco-208 (quinclorac-resistant) and Eco-101 (propanil-, quinclorac-, and imazethapyr-resistant). These two accessions germinated well under cold temperature stress, regardless of biotype, with the lowest GC of Eco-101 being 89% and of Eco-208 being 93% (Table 5.2). These accessions were collected from the northern part of the state where winter temperatures oftentimes are colder, and would remain cold for a longer period, than in the southern part of the state. In the northern Counties, the ten-year average

minimum temperature is 8°C whereas the average minimum temperature is 11°C in southern counties (http://www.usclimatedata.com/climate/arkansas/united-states/3173). Thus, Eco-101 and Eco-208 maybe more cold-acclimated than the southern populations. It also indicates that the resistance mechanism(s) in these two accessions did not cause fitness penalty in GC under cold temperature stress.

Eco-35R2 consistently had greater cumulative germination than Eco-35R1 throughout the duration of the experiment (Table 5.2). This accession was resistant to propanil and quinclorac. The accession with very high single resistance to quinclorac (Eco-76R1) had greater GC than its R2 counterpart. Since the R1 biotype of the single-resistant accession Eco-76 had better germination, the dual resistance to propanil and quinclorac in Eco-35 appeared to have some impact on reduced germination in highly resistant individuals. This could be due to expending more energy to achieve resistance to both herbicides, diverting resources from growth processes. There has been some fitness costs associated with quinclorac resistance in wild mustard (*Brassica arvensis* (L.) Rabenh) that caused plants to be less fit (Jugulam and Goddar 2013). It has been thought that the resistance mechanism for quinclorac was TSR (Grossmann and Kwiatkowski 1995). Further investigation showed when sensitive species like barnyardgrass were exposed to quinclorac, accumulation of cyanide occurred in root and shoot tissues so the mechanism of resistance is not fully understood (Grossmann and Kwiatkowski 2000). This translocation of cyanide throughout the plant could potentially be NTSR. This indicates that although resistance to quinclorac had diverted some carbon resources to produce detoxification or protection enzymes (antioxidants), the upregulation of these proteins had afforded higher stress protection to the developing seedlings in Eco-76R1. Resistance of barnyardgrass/junglerice to quinclorac, alone or in combination with other rice herbicides, has

been documented in the U.S. mid-south since the 1990s (Grossmann K and Kwiatkowski 1995; Lovelace et al. 2007). Because these populations were preselected with propanil (Talbert and Burgos 2007), the predominant multiple resistance pattern is with propanil and quinclorac (Rouse et al. 2018).

Also, at 15 and 20°C, Eco-45R1, Eco-35R1, Eco-225R1, and Eco-245R1 all had significantly less GC or G_{max} than their R2 counterparts; all of these accessions except Eco-245 had resistance to two or more herbicides (Table 5.2-5.3). It would be advantageous for producers to plant cold- tolerant rice varieties that canopy faster to reduce light captured by these highly resistant populations and reduce germination. This also means that in cooler, early-season conditions herbicide applications would kill less R1 individuals; thus, the addition of other PRE herbicides with longer residual in rice production systems is needed to reduce the population size of genotypes similar to R1. Persistent management of resistant junglerice populations across years would reduce the soil seed bank significantly (Egley and Chandler 1983; Gardarin et al. 2010). When looking at both sensitive biotypes of Eco-26, some cold stress tolerance was endowed to Eco-26S1 as it had higher GC than its S2 counterpart at 15°C but not significantly higher (Table 5.3 and 5.4). This accession showed sensitivity to cyhalofop and studies have been conducted looking into ACCase fitness costs. Some resistance-conferring mutations to ACCase herbicides like cyhalofop have been deemed neutral in terms of fitness effects (Leu_{1781}) while one mutation has been associated with reduction in plant fitness (Gly_{2078}) in blackgrass (*Alopecurus myosuroides* (L)) populations from Europe (Menchari et al 2008). The mutation that was associated with reduced fitness was found to occur less frequently in R populations and thus thought to be a recessive trait (Menchari et al. 2006; Darmency et al. 2014). Alternatively, it could be due to undesirable effect of mutations, making the plant unable to compete with other

genotypes in the field. ACCase NTSR mechanisms have been associated with upregulation of certain cytochrome P450 monooxygenase genes, which result in increased detoxification of the ACCase herbicide (Kukorelli et al. 2013). This upregulation of CytP450 genes may also indirectly increase the plant tolerance to other abiotic stresses due to the multiple beneficial functions of CytP450 enzymes.

Estimates of G_{max} from the fitted model were similar to the observed GC at 15-20°C. Both Eco-225R2 and Eco-245R2 had greater G_{max} than their R1 counterparts at lower temperatures, but as temperature increased to 30°C Eco-245R1 had higher G_{max} than its R2 counterpart. At 40°C Eco-225R1 G_{max} was higher than that of Eco-225R2. These two accessions had slower rates of germination when incubated at 15-20°C and did not reach 50% germination at the end of the experiment (Figure 5.1). Both of these accessions are from the warm, southern part of the state; thus, might be more acclimated to higher soil temperatures.

The germination capacity of R1 and R2 biotypes of Eco-101 and Eco-208 were similar at warmer temperatures (Figure 5.2) although slight, non-significant separation was observed at lower temperatures (Figure 5.1). This reflects the fact that junglerice originated from the tropics and although its optimum germination temperature was determined to be around 30-35°C (Chauhan 2009), it can germinate equally well at 40°C. Both of these accessions showed resistance to one or more herbicides and both showed resistance to quinclorac. The germination rate of Eco-76R1 (resistant to quinclorac only) was slightly faster (T_{50} = 10 d) at 15°C than that of Eco-76R2 (T_{50} = 12 d). Eco-76R1 also had higher GC and G_{max} values than Eco-76R2 at this temperature (Table 5.2; Figure 5.1). Quinclorac, being an auxin-type herbicide, has complex activity in plants. It affects many genes; therefore, deciphering resistance mechanism to quinclorac is difficult. The resistance mechanism could be a target site mutation; some

quinclorac-resistant barnyardgrass populations are suspected to have a target site mutation in the auxin-binding site (Grossman and Kwiatkowski 1995; 2000; Lovelace et al. 2007). This hypothesis has not been verified because the specific target is not known. Some TSR mechanisms have minimal to no fitness costs (Sibony and Rubin 2002). Eco-45-R1 had faster germination rates at 15°C with a T_{50} value of 9 days but germination rate levelled off after this, resulting in significantly lower G_{max} values than Eco-45R2 (T_{50}=14 days) (Table 5.2; Figure 5.1).

Conclusions

At high temperatures, between 30 and 40°C, the germination capacity of junglerice is high (>90%) regardless of resistance trait(s) or level of resistance. Single or multiple resistance to propanil, quinclorac, and imazethapyr do not cause fitness penalty in germination behavior of junglerice populations represented in this study under high temperature. It is not certain whether this holds true for all other resistant junglerice populations, which may harbor different resistance mechanisms. Regardless of resistance trait, cold temperature stress delays germination of junglerice and reduces the GC of most populations. High level of multiple resistance to propanil and quinclorac or propanil and imazethapyr seem to lower the GC at cold temperature. Extreme high resistance to quinclorac alone may endow plants with increased germination vigor under cold temperature. All these depend on the resistance mechanism(s) involved. Follow-up research is needed to determine the specific resistance mechanisms in each of these accessions.

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Tables

Table 5.1 *Echinochloa colona* accessions and associated herbicide resistance profiles.

Accession[a]	Resistance profile[b]	Latitude	Longitude
Eco-45	propanil, quinclorac	35°55’28” N	91°09’20.76” W
Eco-101	propanil, quinclorac, imazethapyr	33°39’53” N	91°30’41.28” W
Eco-35	propanil, quinclorac	34°48’04” N	91°06’49.38” W
Eco-208	quinclorac	33° 21’21” N	91°26’0.474” W
Eco-225	propanil, imazethapyr	35°55’28” N	91°09’20.76” W
Eco-245	quinclorac	34°48’04” N	91°06’49.38” W
Eco-26	susceptible to all herbicides	33° 21’21” N	91°26’0.474” W
Eco-76	quinclorac	33°39’53” N	91°30’41.28” W

[a] Accessions were collected between 2012 and 2015 in Arkansas, USA.

[b] Resistance profiles were generated from bioassays of field-collected accessions.

Table 5.2. Germination capacity of *Echinochloa colona* after 26 d of incubation under different temperatures.

Accession[a]	Germination capacity[b]			
	15°C	20°C	30°C	40°C
	(%)			
Eco-45R1	86.9	96.6	96.8	98.7
Eco-45R2	97.0	96.6	98.2	99.6
Eco-101R1	93.5	92.3	89.1	97.9
Eco-101R2	94.2	95.7	90.7	99.0
Eco-35R1	41.7	29.2	66.8	86.4
Eco-35R2	70.0	67.8	86.6	99.0
Eco-208R1	98.9	98.6	97.0	99.2
Eco-208R2	97.7	93.5	99.4	99.7
Eco-225R1	10.3	11.2	82.0	99.9
Eco-225R2	19.4	14.9	71.1	99.9
Eco-245R1	28.1	43.6	89.2	99.6
Eco-245R2	36.7	57.0	85.1	97.2
Eco-26S1	47.1	19.1	94.1	91.5
Eco-26S2	42.2	42.1	79.1	93.1
Eco-76R1	95.3	95.7	89.6	99.9
Eco-76R2	84.1	85.1	90.7	99.9
$LSD_{0.05}$[c]	4.7			

[a]Accessions were collected between 2012 and 2015 in Arkansas, USA; R1 = highly resistant, R2=resistant, S1 = highly sensitive, S2 = sensitive.

[b] Cumulative germination at 26 d of incubation, 12-h photoperiod, constant day/night temperature.

[c] Fisher's protected LSD (α=0.05) to compare accessions and biotype across temperatures.

Table 5.3 Parameter estimates for germination (G_{max}, maximum germination %; T_{50}, days until 50% germination) using three-parameter sigmoid model.

	Parameter estimates							
	15°C		20°C		30°C		40°C	
Accession[a]	G_{max} (%)	T_{50} (days)	G_{max} (%)	T_{50} (days)	G_{max} (%)	T_{50} (days)	G_{max} (%)	T_{50} (days)
Eco-45-R1	82(±2.2)	9(±0.27)	95(±0.4)	4(±0.03)	97(±0.47)	3(±0.04)	100(±0.8)	1(±0.2)
Eco-45-R2	113(±11.1)	14(±1.1)	94(±0.8)	4(±0.06)	96(±0.5)	3(±0.07)	100(±0.4)	1(±0.1)
Eco-101-R1	91(±1.2)	8(±0.3)	89(±0.9)	4(±0.1)	75(±2.4)	3(±0.3)	97(±0.5)	2(±0.06)
Eco-101-R2	91(±1.8)	9(±0.2)	91(±1.1)	4(±0.09)	81(±1.6)	3(±0.2)	98(±1.0)	2(±0.16)
Eco-35-R1	40(±2.0)	10(±0.6)	27(±0.4)	nd[b]	*	16(±8.3)*	84(±0.9)	3(±0.1)
Eco-35-R2	67(±3.2)	11(±0.6)	65(±0.5)	4(±0.2)	79(±1.5)	2(±0.4)	99(±0.3)	1(±0.07)
Eco-208-R1	98(±0.3)	8(±0.4)	98(±0.3)	4(±0.1)	98(±0.4)	3(±0.05)	98(±0.06)	1(±0.0)
Eco-208-R2	94(±2)	8(±0.2)	98(±0.5)	4(±0.1)	100(±0.5)	3(±0.07)	99(±0.0)	2(±0.0)
Eco-225-R1	11(±0.1)	nd[b]	11(±0.3)	nd[b]	*	7 (±6)*	101(±0.9)	5(±0.08)
Eco-225-R2	18(±1.0)	nd[b]	13(±0.9)	nd[b]	*	11 (±7)*	90(±0.5)	3(±0.05)
Eco-245-R1	31(±3.5)	nd[b]	42(±0.2)	nd[b]	95(±20)	9(±3.74)	100(±0.4)	3(±0.04)
Eco-245-R2	38(±2.4)	nd[b]	54(±1.0)	nd[b]	69(±3.3)	3(±0.5)	96(±4.9)	4(±0.6)
Eco-26-S1	44(±1.6)	nd[b]	20(±1.5)	nd[b]	*	16 (±9)*	88(±2.1)	nd[b]
Eco-26-S2	38(±1.2)	nd[b]	40(±0.57)	nd[b]	*	16 (±9)*	91(±1.1)	nd[b]
Eco-76-R1	91(±4.7)	10(±0.6)	93(±3.2)	5(±0.5)	85(±1.3)	3(±0.1)	99(±0.06)	2(±0.008)
Eco-76-R2	85(±5.1)	12(±0.7)	83(±3.7)	6(±0.7)	83(±1.4)	3(±0.1)	99(±0.2)	2(±0.03)

[a] Accessions were collected between 2010 and 2015 in Arkansas, USA. Highly resistant (R1), resistant (R2), sensitive (S1), and highly sensitive (S2) lines were isolated from the field populations via herbicide bioassays.

[b] nd - cannot be determined because germination did not reach 50%.

[c]Numbers in parenthesis are one standard error of the estimate.

* The three-parameter sigmoid model did not fit the data. The data was best described by a quadratic polynomial function f=y0+a*x+b*x^2.

Table 5.4 Summary of differentiation between resistant resistant biotypes in germination capacity and G_{max} across various temperatures.

	15°C		20°C		30°C		40°C	
	R1 > R2[c]	R1 < R2[c]	R1 > R2[c]	R1 < R2[c]	R1 > R2[c]	R1 < R2[c]	R1 > R2[c]	R1 < R2[c]
Germination capacity[a]								
	Eco-76	Eco-35	Eco-76	Eco-35	Eco-225	Eco-35		Eco-35
		Eco-45		Eco-245				
		Eco-225						
		Eco-245						
G_{max}[b]								
	Eco-208	Eco-35	Eco-76	Eco-35	Eco-245	Eco-101	Eco-225	Eco-35
		Eco-45		Eco-225				
		Eco-225		Eco-245				
		Eco-245						

[a] The total number of seeds that germinated at termination of the study relative to the total number of seeds incubated.
[b] G_{max} is the estimate of maximum germination from the three-parameter sigmoid function.
[c] R1>R2, R1<R2 indicate whether highly resistant (R1) plants germinated more, or less, than the resistant (R2) plants.

Figures

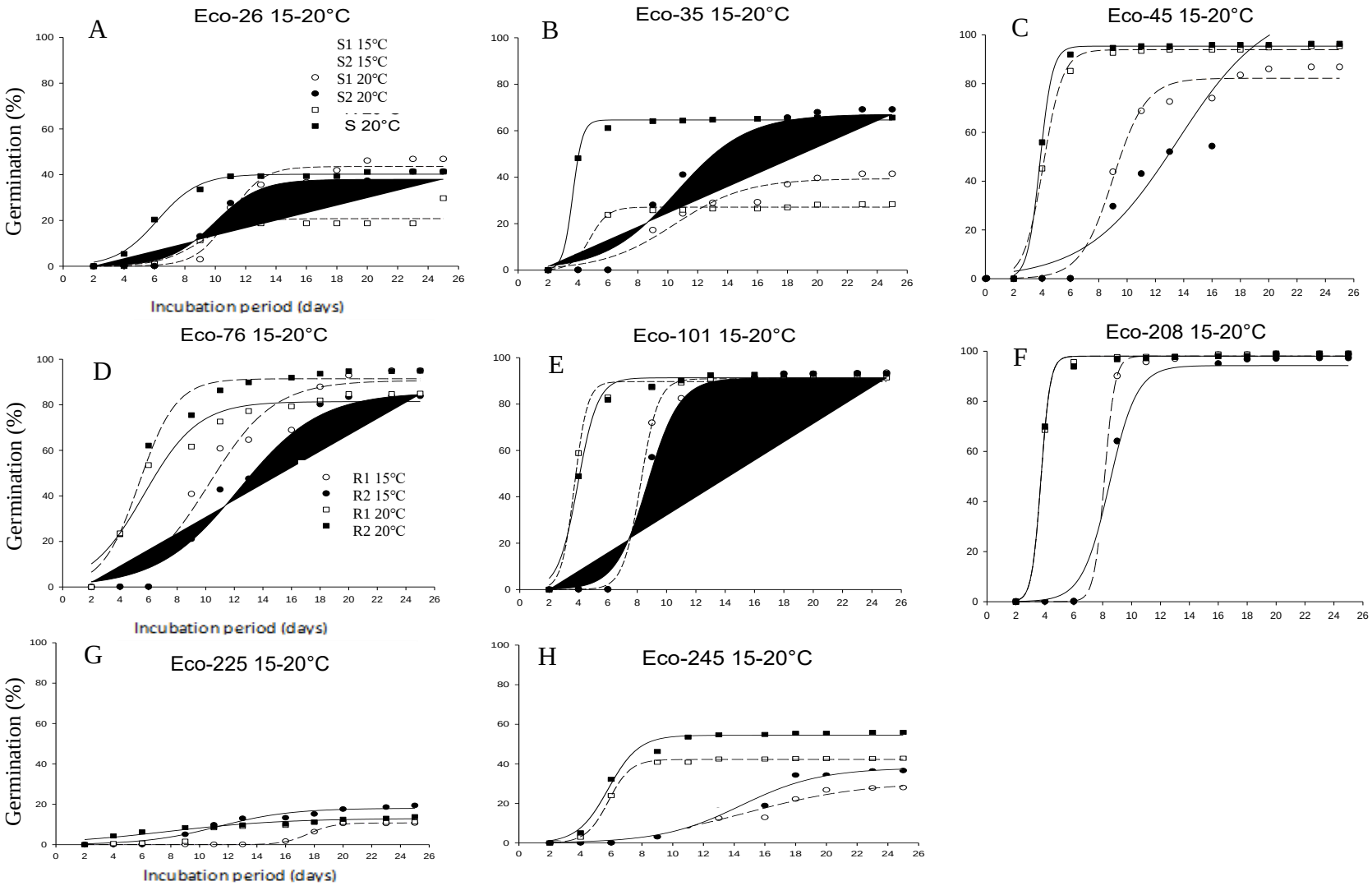

Figure 5.1 (a-h). Effect of temperature (15 and 20°C) on germination of *Echinochloa colona* with various herbicide resistance profiles.

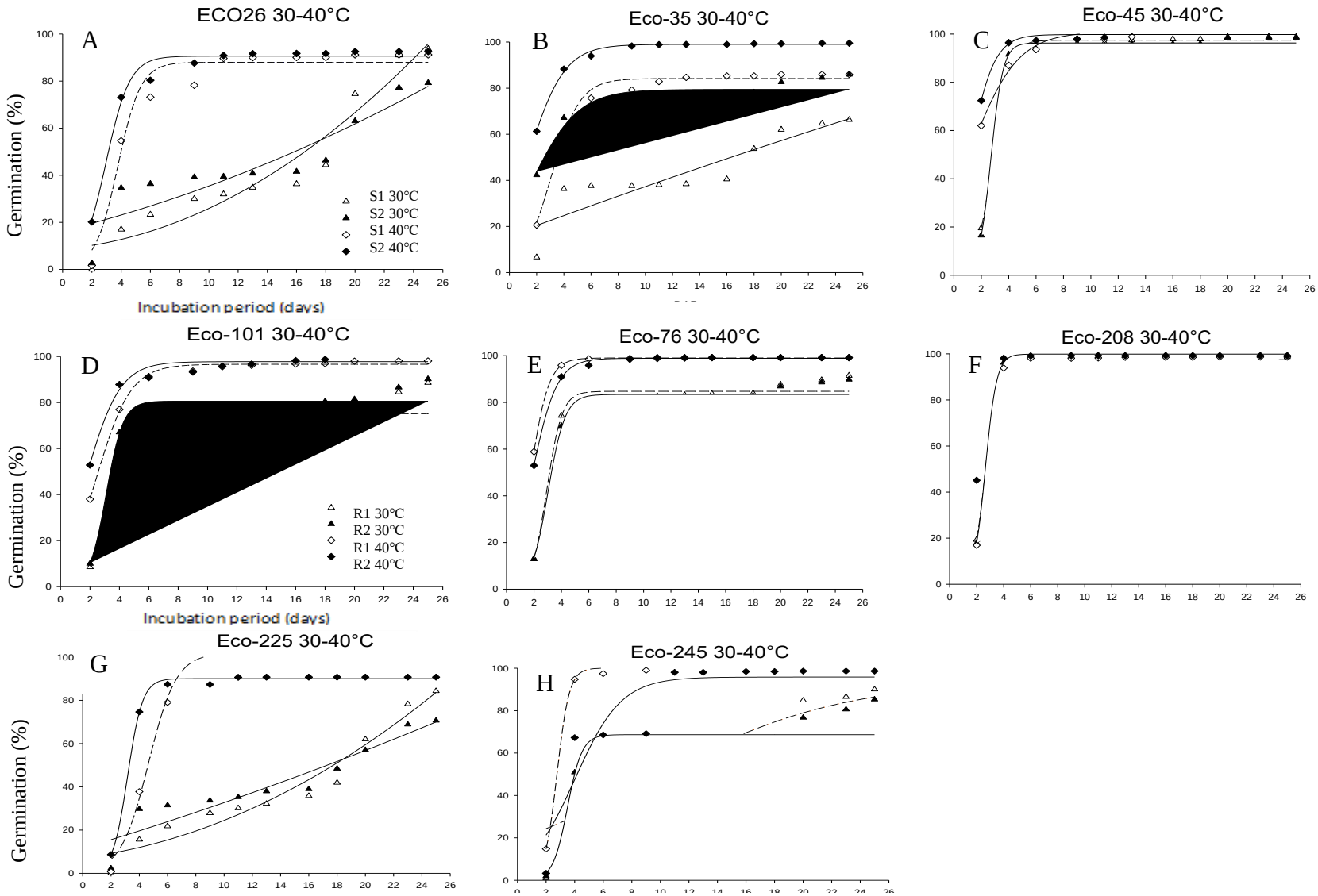

Figure 5.2 (a-h). Effect of temperature (30 and 40°C) on germination of *Echinochloa colona* with various herbicide resistance profiles.

Chapter 6: Conclusion

Dose response assays showed that attempts to segregate S and R biotypes from junglerice populations resulted in biotypes that were highly resistant and resistant. These biotypes had no fitness penalties when grown in monocultures under optimum conditions. Accessions with biotypes that showed differences in injury response also exhibited differences in germination under cold-stressed environments. In replacement studies, both single- and multiple-resistant junglerice out-competed rice; however, the single resistant accession competed with rice more than the multiple resistant accession. Since there were no differences in response between biotypes of either accession, the heightened competition of the single resistant accession is due to morphological and background evolution traits.