PARAQUAT-RESISTANT ITALIAN RYEGRASS (Lolium multiflorum) CONFIRMED IN LOUISIANA

Alphonse B. Coco III
*Louisiana State University and Agricultural and Mechanical College*

Follow this and additional works at: https://repository.lsu.edu/gradschool_theses

Part of the Agriculture Commons, and the Plant Sciences Commons

**Recommended Citation**
https://repository.lsu.edu/gradschool_theses/5693

This Thesis is brought to you for free and open access by the Graduate School at LSU Scholarly Repository. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Scholarly Repository. For more information, please contact gradetd@lsu.edu.
PARAQUAT-RESISTANT ITALIAN RYEGRASS (Lolium multiflorum) CONFIRMED IN LOUISIANA

Alphonse B. Coco III

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

Part of the Agriculture Commons, and the Plant Sciences Commons
PARAQUAT-RESISTANT ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM*) CONFIRMED IN LOUISIANA

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in The School of Plant, Environmental, and Soil Sciences

by
Alphonse B Coco III
B.S., Louisiana Technical University, 2016
December 2022
Acknowledgements

There are so many people I would like to thank for their assistance, direction, and aid during the course of this experiment. First and foremost, I would like to thank Dr. Al Orgeron, without whom, none of this would have happened. Working with you encouraged me to continue my education, and you helped and guided me through every step of our research. For that, I cannot thank you enough. To Dr. Doug Spaunhorst and Dr. Lauren Lazaro, thank you for sharing your facilities and equipment with us; your generosity enabled us to ensure a statistical consistency and accuracy we otherwise would have not been able to achieve. To Dr. Michael Pontiff, Brayden Blanchard, Zach Taylor, and the entire breeding program at the LSU AgCenter Sugar Research Station, thank you for sharing your space and resources with me to perform the bulk of my work onsite. All of you played a significant part in my research being completed, and I cannot thank you enough.

To my family, Alphonse and Mary Coco and Katherine Coco, without your experiences in the agricultural field, technical writing, and unconditional support every step of the way, I could not have accomplished all I have up until now. You helped me grow into who I am today, and I will always be grateful.
To my amazing wife, Caitlynn Coco, you have been my strongest support, encouraging me when I was discouraged, helping me stay on track when life was chaotic, and reminding me to keep my eyes focused on the goal the entire way. I do not know where I would be without you.
Table of Contents

Acknowledgements ........................................................................................................ ii

List of Tables & Figures..................................................................................................... v

Abstract .......................................................................................................................... vi

Chapter 1. Introduction...................................................................................................... 7
   Weed Pest of Sugarcane ................................................................................................. 7
   Italian ryegrass .............................................................................................................. 9
   Herbicide Resistance................................................................................................... 12

Chapter 2. Introduction.................................................................................................... 14
   Material and Methods ................................................................................................. 17
   Results and Discussions ............................................................................................ 20

Chapter 3. Summary........................................................................................................ 30

References ..................................................................................................................... 32

Vita ................................................................................................................................. 36
List of Tables & Figures

Table 1. Location of sugarcane fields where Italian ryegrass seeds were collected ..................22
Table 2. Paraquat rates used in dose-response experiment .................................................................24
Table 3. Control of four Italian ryegrass populations 18 d after treatment with several paraquat rates. ...........................................................................................................................................................................24
Table 4. Dose-response curve regression parameter estimates for visual injury ratings at 18 d after application and dry biomass at 22 d after application of four Italian ryegrass biotypes used in the paraquat dose-response experiment. ...........................................................................................................................................................................27
Figure 1. Dose-response curves of Italian ryegrass control 18 d after treatment in response to increasing rates of paraquat ...........................................................................................................................................................................22
Figure 2. Dose-response curves of Italian ryegrass biomass 22 d after treatment in response to increasing rates of paraquat ...........................................................................................................................................................................24
Abstract

Paraquat is the only herbicide labeled for postemergence control of Italian ryegrass in sugarcane. In 2017, sugarcane farmers in Louisiana reported Italian ryegrass control failures with paraquat applications. Seeds were collected from sugarcane fields in White Castle, St. Gabriel, and Bunkie, Louisiana where control was not achieved (reduced susceptibility). Additionally, Italian ryegrass seeds were collected from a sugarcane field in Welcome, LA with a history of paraquat use and with no reported control issues (susceptible population). A dose-response study was conducted at the Louisiana State University Agricultural Center’s Sugar Research Station in February 2021 and was repeated in November 2021. Paraquat was applied to 8- to 12- cm tall Italian ryegrass plants at 0, 1/32 1/16, 1/8, 1/4, 1/2, 1, 2, 4, 8, and 16X rates, of the maximum field use rate of 840 g ha$^{-1}$, for the reduced susceptibility populations. For the susceptible population, paraquat was applied at 0, 1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, 1, and 2X rates. At 18 d after application, injury was recorded, then biomass was harvested and dried for 96 h. Dose-response curves were fitted using a four-parameter, log-logistic model in R software. The effective paraquat dose to cause 50% injury (ED$_{50}$) was 77 g ha$^{-1}$ for the Welcome population, whereas it required 200.4, 22.2, and 45.4 -fold more paraquat for the White Castle, St. Gabriel, and Bunkie populations, respectively. Likewise, the ED$_{50}$ value required to reduce the dry weight by 50% was 44 g ha$^{-1}$ for the Welcome population. To reduce dry weight biomass for 50% for the White Castle, St. Gabriel, and Bunkie populations, required rates of 13.3, 4.3, and 14.3 -fold more paraquat, respectively, when compared to the Welcome population. These results confirm the presence of paraquat-resistant Italian ryegrass in Louisiana.
Chapter 1. Introduction

In 2021, Sugarcane (Saccharum spp.) was produced on 205,580 hectares in 25 Louisiana parishes (Gravois 2022). Louisiana’s 11 sugar factories processed over 15 million short tons of raw sugarcane, yielding 1.83 million short tons of sugar (96° pol) in the 2021 season (Gravois 2022). Sugarcane is a perennial crop, and several crops are harvested from a single planting. When sugarcane yield becomes unprofitable, fields are plowed out, fallowed and preparations made for planting in August-September. Louisiana’s climate is hot and humid, with 9 months of the year having temperatures averaging 30°C, while the winter months average 17°C but can drop below 10°C. In terms of rainfall, the southern parishes, where most sugarcane is grown, average 178 cm of annual precipitation. Louisiana’s long, hot summers and brief but cold winters, cause the sugarcane shoots that sprout after harvest to be killed back by frost. These environmental conditions provide a window for weeds to become established and compete in sugarcane fields.

Weed Pest of Sugarcane

A great deal of time and capital is spent managing annual and perennial summer weeds in Louisiana. Johnsongrass (Sorghum halepense) is a perennial grass and is highly competitive when not managed and can reduce sugar and cane yield by 30% or more (Ali et al. 1986; Millhollon 1970). Johnsongrass is a prolific weed, common throughout North America, Europe, Africa, Brazil, and Argentina, and southwestern Asia and northern Australia (Howard 2004). It flowers throughout the summer and into fall, reproducing by both seed and rhizomes (Anonymous 2020b). Itchgrass (Rottboellia cochinchinensis) and bermudagrass (Cynodon dactylon) are also major weeds in Louisiana sugarcane production. Millhollon (1992) found that
cane yield was reduced by as much as 19% when itchgrass was removed 180 d after interference, with further reductions in cane yield for the subsequent crop.

Richard and Dalley (2007) reported that bermudagrass competition reduced sugarcane stalk population by 13 to 25% in plant cane and sugar yield by 8 to 32%; however, subsequent stubble cane crops only had reductions of 10% in stalk number and sugar yield. They surmised that subsequent crops fared better due to bermudagrass’ decumbent growth habit and bermudagrass’ low tolerance for shade.

Multiple species of morningglories are found in sugarcane fields in Louisiana as well. Red morningglory (*Ipomoea coccinea*) is one of the most problematic of the morningglory species in sugarcane. It competes with the sugarcane by climbing and entangling the cane, reducing millable stalk population and sugar yield by 30% (Viator et al. 2002). Besides red morningglory, pitted morningglory (*Ipomoea lacunosa*) and entireleaf morningglory (*Ipomoea hederacea*) are also problematic weed species (Jones and Griffin 2008; Millhollon 1988).

Purple nutsedge, another significant weed pest in sugarcane, is a small but rapidly growing weed species that is incredibly resource competitive. Present in over 92 countries, it is a significant threat to several crops, including cotton, corn, and rice, and is capable of reducing cane yield by 75% and sugar yield by 65% (Urbatsch 2019). Etheridge (2007) performed interference and shade studies on purple nutsedge in sugarcane. He found at a density of 4 purple nutsedge tubers per pot, sugarcane shoot and root dry weight of the four sugarcane cultivars selected for this study were reduced from 21 to 71%.

Unlike other sugarcane industries, winter weeds pose a threat to sugarcane production in Louisiana. Freezes are a common occurrence in December, January, and February, which slows sugarcane re-emergence and growth. Winter weeds take advantage of this dormant period,
potentially overtaking fields. Problematic grasses include Italian ryegrass (*Lolium multiflorum*), annual bluegrass (*Poa annua*), rescuegrass (*Bromus catharticus*), and timothy canarygrass (*Phalaris angusta*). Problematic broadleaf weeds, include bur clover (*Medicago polymorpha*), black medic (*Medicago lupulina*), vetch (*Vicia*), buttercup (*Ranunculus* sp.), chickweed (*Stellaria media*), common dandelion (*Taraxacum officinale*), cressleaf groundsel (*Packera glabella*), curly dock (*Rumex crispus*), cutleaf eveningprimrose (*Oenothera laciniata*), Carolina geranium (*Geranium carolinianum*), henbit (*Lamiun amplexicaule*), marestail (*Erigeron canadensis*), purslane speedwell (*Veronica peregrina*), shepherd’s purse (*Capsella bursa-pastoris*), smallflower bittercress (*Cardamine parviflora*), sowthistle (*Sonchus* sp.), swinecress (*Coronopus didymus*), Virginia pepperweed (*Lepidium virginicum*), lambsquarter (*Chenopodium album*), and field bindweed (*Convolvulus arvensis*). These grasses and weeds are commonly found in sugarcane fields throughout Louisiana (Anonymous 2020a; Griffin et al. 2001; Richard and Dalley 2007).

**Italian ryegrass**

Italian ryegrass is a winter weed that presents as both an annual and biennial plant. Under a meter in height, Italian ryegrass grows in a bunch, with nodes and internodes present on stems. Leaves form in a bunch at the base of the plant, then the nodes each support a leaf, which is hairless and grows in an alternating pattern. Auricles are present on the stems, which can aid in identification. Italian ryegrass produces a spiked seedhead at the top of each stem on the plant, which averages 30 cm in length, with each spikelet following an alternating pattern (Anonymous 2020c). Because of an interaction between 2 loci with multiple alleles, Italian ryegrass produces short pollen tubes. This in turn renders Italian ryegrass incapable of self-pollinating; therefore, ryegrass outcrosses with other Italian ryegrass plants or other ryegrass species (Fearon et al.
1983). Additionally, Italian ryegrass pollen is easily dispersed by the wind which aids outcrossing events (Jhala et al. 2021).

Italian ryegrass is a problematic weed in winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and alfalfa (*Medicago sativa* L.) production. It is highly competitive with winter wheat, specifically during the reproductive stages (Hashem et al. 1998). Reductions in yield have been recorded from 60 to 92%. Liebl and Worsham (1987) estimated a 4.2% reduction in wheat yield per 10 Italian ryegrass plants m$^{-2}$. Italian ryegrass decreased the number of tillers that wheat produced, resulting in reduced grain yield.

Burndown is a common agricultural practice where herbicides are applied to emerged weeds to prepare the field for planting. Soybean crops have shown an increase of soybean yield of nearly 2,300 kg ha$^{-1}$ when a glyphosate-based burndown program is used prior to planting (Eubank et al. 2008).

Herbicide strategies for managing Italian ryegrass include the use of PRE and POST herbicide applications to prevent the establishment of new plants and control emerged plants. Flufenacet combined with metribuzin is a common tank mix herbicide treatment to control Italian ryegrass in wheat fields in Oregon (Liu et al. 2016). When flufenacet-resistance started becoming a concern, alternative herbicides were considered, such as pyroxasulfone. Pyroxasulfone and flufenacet are both Group 15 herbicides, which inhibit long-chain fatty acids.

Bond et al. (2014) conducted an experiment in Mississippi on glyphosate-resistant Italian ryegrass to evaluate the effectiveness of clomazone. Clomazone applied at 0.84 kg ha$^{-1}$ at 3 application timings (September, October, and November) reduced dry weight yields by 80-100% 35 d after the final treatment in November and 44-100% 140 days after the last treatment in
November. The highest level of control with clomazone was achieved with the November application, whereas the poorest control occurred with the September application. Bond et al. (2014) also showed that incorporated pendimethalin and trifluralin, applied at 1.59 kg ha\(^{-1}\) and 1.68 kg ha\(^{-1}\) respectively, provided over 90% control of Italian ryegrass versus surface application. Additionally, he reported S-metolachlor and pyroxasulfone also provided over 90% control of Italian ryegrass.

PRE management of weeds in sugarcane can utilize several different herbicides for both grass and broadleaf weeds. Metribuzin, S-metolachlor, pendimethalin, sulfentrazone, and atrazine are herbicides commonly used to control grass and broadleaf weeds in sugarcane (Anonymous 2020a; Orgeron 2020).

POST herbicides such as diclofop-methyl, quizalofop, clethodim, pinoxaden, pyroxsulam, imazapyr, imazamox, glyphosate, and paraquat can be used to control Italian ryegrass (Anonymous 2020a; Bond et al. 2005; Griffin 2004). However, Italian ryegrass has developed resistance to many of these herbicides (Betts et al. 1992; Bond et al. 2005; Ellis et al. 2008; Liu et al. 2016; Nandula et al. 2007).

POST management of emerged Italian ryegrass in sugarcane is limited to paraquat and can safely be applied to sugarcane at 840 g ha\(^{-1}\) up to the 4-leaf stage without impacting sugarcane yield (Griffin and Judice 2009). Paraquat is classified as a photosystem I (PS I) herbicide. PS I herbicides act upon plants by interrupting and diverting the flow of electrons that results from chlorophyll absorbing sunlight and would then be sent through Photosystem I. These electrons react with divalent paraquat cations to produce free radicals that then oxidize with oxygen and water, rapidly destroying the cell membrane, which leads to mass cell death (Anonymous 2020d; Chiang et al. 2008). Paraquat is a contact herbicide, and direct application
to the target plant’s leaves is required to maximize efficacy. Once in the plant paraquat does not readily translocate to meristematic tissues, and soil contact renders it inert (Anonymous 2020d).

Paraquat is highly toxic to mammals when ingested. Paraquat attacks electron transfer in mammals via the mitochondria and is equally destructive (Fuerst and Vaughn 1990). Because of cases of accidental ingestion and poisoning from paraquat, the EPA required modifications to the paraquat use label in 2019. Specific changes were made regarding the handling, usage, and application of paraquat to highlight the danger and lethality of improper handling. Applicators and handlers of paraquat must be certified licensed pesticide applicator and must gain additional online training to become certified (Cato 2019).

Herbicide Resistance

Herbicide resistance is a genetic mutation that can occur spontaneously or in response to repeated exposure of herbicide and can have severe effects on agricultural practices and crop yield. Commercialized in 1974, glyphosate is the most widely used herbicide due to its nonselective activity and systemic nature (Jasieniuk et al. 2008). Now, almost 50 years later, glyphosate-resistance in numerous weed species is a concern. To date, there are 41 confirmed species of glyphosate-resistant weeds across the world (Heap 2022). The first reported case of glyphosate resistance was in rigid ryegrass in Australia, 1996 (Perez-Jones et al. 2005).

In 1987, Italian ryegrass first developed resistance to diclofop-methyl, an ACCase (Acetyl-CoA carboxylase) herbicide in Oregon, and again in North Carolina in 1990 (Betts et al. 1992; Bond et al. 2005). ACCase herbicides inhibit fatty acid synthesis, as well as membrane synthesis. Diclofop-methyl is applied foliarly and translocates throughout the plant, causing plant death in 2-3 weeks. Italian ryegrass has since developed resistance to many herbicides including: glyphosate, glufosinate, quizalofop, clodinafop, clethodim, tralkoxydim, pinoxaden, pyroxsulam,
imazapyr, imazamox, mesosulfuron-methyl, sulfometuron, flucarbazone, triasulfuron, flufenacet, atrazine, diuron, hexazinone, oxyfluorfen, and amitrole have been documented in populations of (Boba dilla et al. 2021; Bond et al. 2005; Bond et al. 2014; Ellis et al. 2008; Fernández et al. 2017; Ghanizadeh et al. 2015; Heap 2022; Lui et al. 2014; Lui et al. 2016; Nandula et al. 2007; Nandula et al. 2019; Rauch et al. 2010).

In 2015, Italian ryegrass seed collected from orchards, field crops, and roadsides in California’s Central Valley revealed resistance to paraquat in two populations (Tehranchian et al. 2018). Tehranchian et al. (2018) work showed these populations were resistant to multiple herbicides including paraquat, glyphosate and sethoxydim. Likewise, paraquat-resistant Italian ryegrass was confirmed in a prune orchard in Hamilton City, CA (Brunharo and Hanson 2018).

In 2017, some sugarcane farmers in southern Louisiana began reporting that labeled rates of paraquat were not providing adequate control of Italian ryegrass. Paraquat is the only labeled herbicide for controlling emerged Italian ryegrass in sugarcane. The purpose of this research was to evaluate the suspected resistance of several Italian ryegrass populations in commercial sugarcane fields in Louisiana.
Chapter 2. Introduction

Sugarcane (*Saccharum* spp.) is a tropical perennial grass that is commercially grown in the states of Louisiana, Florida, and Texas. In 2021, Louisiana sugarcane production exceeded 205,500 hectares and was the state’s most valuable row crop (Gravois 2022). Sugarcane is a vegetatively propagated crop, and whole stalks or stalk segments are planted into raised beds in late July through September in Louisiana. Sugarcane is a perennial crop, and a single planting event is typically harvested for four or more years. Harvest in Louisiana occurs from late-September to mid-January, and sugarcane is processed into table sugar (sucrose). Other byproducts of the milling process include molasses and bagasse, which are used for animal feed stock and as a fuel source to power boilers within the mills, respectively. Sugarcane often reemerges slowly following harvest due to cold temperatures and freeze events which occur from November through early-March, thus limiting above ground biomass.

While weeds which thrive under hot conditions such as johnsongrass (*Sorghum halepense*), itchgrass (*Rottboellia cochinchinensis*), and bermudagrass (*Cynodon dactylon*) are common to sugarcane production in Louisiana and other growing regions, winter weeds are more problematic in Louisiana. Winter weeds take advantage of sugarcane’s slow growth during the winter months, potentially overtaking fields when left unmanaged. Both grasses, including Italian ryegrass (*Lolium multiflorum*), annual bluegrass (*Poa annua*), rescuegrass (*Bromus catharticus*), and timothy canarygrass (*Phalaris angusta*) and many broadleaf weeds are common to Louisiana sugarcane production.

PRE management of winter weeds in sugarcane has historically been accomplished with metribuzin, diuron, or atrazine. *S*-metolachlor was first labeled for U.S. sugarcane production in 2018 as a premix in combination with atrazine and mesotrione (Lumax® EZ), and additional
standalone formulations of S-metolachlor were registered for use in sugarcane in 2020. Orgeron (2021) reported that S-metolachlor was an excellent control option for PRE control of Italian ryegrass.

Italian ryegrass germinates throughout the winter period and can be found throughout Louisiana’s cropping systems (Miller et al. 2002). While glyphosate or ACCase herbicides can be utilized in fallow situations to manage Italian ryegrass POST, such applications in-crop would kill or severely injure sugarcane. POST management of Italian ryegrass in sugarcane is limited to paraquat and can safely be applied to sugarcane at 840 g ha\(^{-1}\) up to the 4-leaf stage without impacting sugarcane yield (Griffin and Judice 2009). Paraquat is a contact herbicide, and direct application to the target plant’s leaves is required to maximize efficacy. Paraquat is a photosystem I herbicide and works by interrupting and diverting the flow of electrons that results from the chlorophyll absorbing sunlight and would then be sent through Photosystem I. These electrons react with divalent paraquat cations to produce free radicals that then oxidize with oxygen and water, rapidly destroying the cell membrane, which leads to mass cell death (Anonymous 2020; Chiang et al. 2008).

In 2017, Italian ryegrass control failures with paraquat were reported in some sugarcane fields within the Louisiana industry. One possible explanation for the control failures was the development of herbicide resistance. Herbicide resistance is a genetic mutation that can occur spontaneously or in response to repeated exposure of herbicide and can have severe negative control effects on agricultural practices and yields. Resistance is a heritable trait passed from one plant to its offspring that allows it to survive and reproduce after exposure to higher rates of herbicide than the baseline population.
In 1987, Italian ryegrass developed resistance to diclofop-methyl in Oregon (Betts et al. 1992). Since then, resistance to glyphosate, glufosinate, quizalofop, clodinafop, clethodim, tralkoxydim, pinoxaden, pyroxsulam, imazapyr, imazamox, mesosulfuron-methyl, sulfometuron, flucarbazone, triasulfuron, flufenacet, atrazine, diuron, hexazinone, oxyfluorfen, and amitrole have been documented in populations of Italian ryegrass (Bobadilla et al. 2021; Bond et al. 2005; Bond et al. 2014; Ellis et al. 2008; Fernández et al. 2017; Ghanizadeh et al. 2015; Heap 2022; Lui et al. 2014; Lui et al. 2016; Nandula et al. 2007; Nandula et al. 2019; Rauch et al. 2010).

Preliminary screening of glyphosate-resistant Italian ryegrass populations collected in 2015 from orchards, field crops, and roadsides in California’s Central Valley revealed resistance to paraquat in two populations (Tehranchian et al. 2018). This work confirmed multiple herbicide resistance to paraquat as well as glyphosate and sethoxydim to Italian ryegrass collected from an almond orchard and alfalfa field in Glenn County California (Tehranchian et al. 2018). Likewise, Brunharo and Hanson (2018) confirmed a case of Italian ryegrass with multiple herbicide resistance, including paraquat as well as glyphosate and clethodim, in a prune orchard in Hamilton City, CA. More recently, paraquat-resistant Italian ryegrass has been confirmed in hazelnut orchards in western Oregon (Moretti 2021).

Since 1980, 31 weed species have populations that have been confirmed as paraquat-resistant and exist in 20 countries (Heap 2022). The first weed in the United States to be documented as resistant to paraquat was American black nightshade (Solanum americanum) in 1985 (Heap 2022). Since then, paraquat resistance has been documented in several weeds including horseweed (Coryza canadensis), goosegrass (Eleusine indica), duckweed (Landoltia punctata), hairy fleabane (Coryza bonariensis), and Italian ryegrass (Lolium multiflorum) (Heap 2022).
While other row cropping systems have several herbicide chemistries which can be utilized to manage Italian ryegrass POST, sugarcane is limited to paraquat. Recent control failures are a concern for the Louisiana sugarcane industry. The objective of this study was to determine the sensitivity of Italian ryegrass populations collected from commercial sugarcane fields in Louisiana to applications of paraquat.

**Materials and Methods**

An outside container experiment was conducted at the LSU AgCenter’s Sugar Research Station in Saint Gabriel, Louisiana to evaluate the sensitivity of Italian ryegrass populations to paraquat. In 2018, seed for the experiment were collected from multiple plants from the same field from sugarcane fields at three different locations in Louisiana where reduced paraquat susceptibility was reported (White Castle or R1, St. Gabriel or R2, and Bunkie or R3) at the maximum field application rate of 840 g ha⁻¹, and one commercial sugarcane field in Louisiana with no reported paraquat susceptibility issue (Welcome or S1) (Table 1.).

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Welcome</td>
<td>30.05498 90.93253</td>
</tr>
<tr>
<td>R1</td>
<td>White Castle</td>
<td>30.15739 91.10220</td>
</tr>
<tr>
<td>R2</td>
<td>St. Gabriel</td>
<td>30.26665 91.10112</td>
</tr>
<tr>
<td>R3</td>
<td>Bunkie</td>
<td>30.96741 92.16913</td>
</tr>
</tbody>
</table>

Seed from each population were planted into trays (51 cm by 26 cm) filled with BM2® seed germination and propagation mix (Berger, Saint-Modeste, Quebec) medium on November 17, 2020 and October 15, 2021. Trays were then re-located outside onto benches so that plants
were exposed to natural conditions. Trays were watered daily. On December 8, 2020, 21 d after planting, and October 20, 2021, 5 days after planting, 80 seedlings from each population were transplanted into Cone-tainers™ (Stuewe and Sons, Tangent, OR) containing the same BM2® medium as the trays. Once transplanted, the Cone-tainers™ trays were then placed on outside benches to continue growth under ambient environmental conditions. Plants were watered as needed and fertilized with 20-20-20 N-P-K fertilizer (Peters’ Professional 20-20-20 General Purpose Fertilizer, The Scotts Company, Marysville, OH) three weeks after transplanting. The first experimental run was conducted on February 4, 2021, 59 d after transplanting, and the second run was conducted on November 16, 2021, 25 d after transplanting. At treatment, plants averaged 8- to 12-cm in height and had two to five tillers per plant. Treatments were applied with a DeVries Manufacturing Generation 3 Tracked Sprayer with an 8002E nozzle at 187 L ha⁻¹ at 241 kPa to ensure uniform application.

The experiment was designed as a completely randomized design with a factorial arrangement of treatments and treatments were replicated five times per population. The factors were Italian ryegrass population (R1, R2, R3, S1) and paraquat (Gramoxone SL 2.0®, Syngenta Crop Protection, Greensboro, NC) dosage at ten rates (Table 2). Paraquat dosage for the susceptible population was applied at 0, 1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, 1, and 2X of the maximum field use rate of 840 g ha⁻¹, and at 0, 1/32 1/16, 1/8, 1/4, 1/2, 1, 2, 4, 8, and 16X for the reduced susceptibility populations. Nonionic surfactant was added to each treatment at 0.25% v/v (Induce®, Helena Agri-Enterprises LLC, Collierville, TN). Following treatment, plants were maintained in a greenhouse and watered regularly. Injury ratings were recorded 18 d after application based on a scale of 0 to 100% with 0 = no control and 100 = plant death. Plant
biomass was harvested by cutting plants 1.5 cm above the soil surface. The cuttings were
bagged, labeled, and placed in an oven drier at 50° C for 96 h to determine plant dry weight.

Table 2. Paraquat rates used in dose-response experiment.

<table>
<thead>
<tr>
<th></th>
<th>Italian Ryegrass Population</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Welcome (S1)</td>
<td>White Castle (R1)</td>
<td>St. Gabriel (R2)</td>
<td>Bunkie (R3)</td>
</tr>
<tr>
<td>Paraquat</td>
<td>g ha⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.3</td>
<td>26.3</td>
<td>26.3</td>
<td>26.3</td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td>52.5</td>
<td>52.5</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td>13.1</td>
<td>105</td>
<td>105</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>26.3</td>
<td>210</td>
<td>210</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>52.5</td>
<td>420</td>
<td>420</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>840</td>
<td>840</td>
<td>840</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>1,680</td>
<td>1,680</td>
<td>1,680</td>
<td></td>
</tr>
<tr>
<td>420</td>
<td>3,360</td>
<td>3,360</td>
<td>3,360</td>
<td></td>
</tr>
<tr>
<td>840</td>
<td>6,720</td>
<td>6,720</td>
<td>6,720</td>
<td></td>
</tr>
<tr>
<td>1,680</td>
<td>13,440</td>
<td>13,440</td>
<td>13,440</td>
<td></td>
</tr>
</tbody>
</table>

*a Gramoxone 2SL®, Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC.

Statistical Analysis

Data were analyzed using a nonlinear regression in R software v 1.0.143 using the dose response curve (drc) package (Ritz et al. 2015). After evaluating various models, the 4-parameter log-logistic model (Seber and Wild 1989) was utilized to relate the percent control and dry weight over paraquat rates.

\[
Y = c + \frac{d - c}{1 + \exp\left(b \log(x) - \log(e)\right)}
\]

In this model, \(y\) is the response variable, \(c\) is the lower limit of \(y\), \(d\) is the upper limit of \(y\), \(b\) is the slope of the curve around the ED\(_{50}\), \(x\) is the herbicide dose, and \(e\) is the ED\(_{50}\) and is the paraquat rate needed to provide 50% control or reduce dry weight by 50%. To calculate the differential sensitivity among the populations to paraquat, each reduced susceptibility population was divided by the susceptible population (S1).
Results and Discussion

The maximum labeled field rate of paraquat, 840 g ha\(^{-1}\) (1X rate), provided 100% control of the Welcome (S1) population 18 d after treatment, whereas control averaged 25, 30, and 28% for the White Castle (R1), St. Gabriel (R2), and Bunkie (R3) populations, respectively (Table 3.) In a similar dose response evaluation of paraquat, Tehranchian et al. (2018) reported complete control of the susceptible Italian ryegrass population (collected from a vineyard in Sonoma County California) with paraquat at 70 g ha\(^{-1}\). He also achieved a high level of control of the MR1 (collected from an almond orchard in Glenn County California) population and complete control of the MR2 (collected from an alfalfa field in Glenn County California) population of Italian ryegrass with paraquat at 1,121 g ha\(^{-1}\).

Table 3. Control of four Italian ryegrass populations 18 d after treatment with several paraquat rates.

<table>
<thead>
<tr>
<th>Paraquat(^{a}) Rate g ha(^{-1})</th>
<th>Italian Ryegrass Population</th>
<th>% control(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Welcome (S1)</td>
<td>White Castle (R1)</td>
<td>St. Gabriel (R2)</td>
</tr>
<tr>
<td>26.3</td>
<td>10 g-j(^{c})</td>
<td>6 ij</td>
</tr>
<tr>
<td>52.5</td>
<td>27 ef</td>
<td>5 ij</td>
</tr>
<tr>
<td>105</td>
<td>70 b</td>
<td>15 f-j</td>
</tr>
<tr>
<td>210</td>
<td>85 ab</td>
<td>19 f-i</td>
</tr>
<tr>
<td>420</td>
<td>95 a</td>
<td>29 ef</td>
</tr>
<tr>
<td>840</td>
<td>100 a</td>
<td>25 e-g</td>
</tr>
<tr>
<td>1680</td>
<td>100 a</td>
<td>52 c</td>
</tr>
</tbody>
</table>

\(^{a}\)Gramoxone 2SL\(^{®}\), Syngenta Crop Protection, P.O. Box 18300, Greensboro, NC.

\(^{b}\)Control based on a scale of 0 to 100% with 0 = no control and 100% = plant death.

\(^{c}\)Means followed by the same letter are not significantly different (P ≤0.05).

Italian ryegrass control was modeled as a function of paraquat rate using a four-parameter log-logistic model and the lack of fit test was not significant (P > 0.05). The ED\(_{50}\) value for visual control averaged 77 g ha\(^{-1}\) for the Welcome population, whereas the ED\(_{50}\) for the White
Castle, St. Gabriel, and Bunkie populations averaged 15,482, 1,713, and 3,510 g ha\(^{-1}\), respectively (Figure 1 and Table 4).

Figure 1. Dose-response curves of Italian ryegrass control 18 d after treatment in response to increasing rates of paraquat. Plants were treated at 8- to 12- cm in height (two to five tillers). Dose-response curves were fitted using a four-parameter, log-logistic model in R software.
Table 4. Dose-response curve regression parameter estimates for visual injury at 18 d after application and dry biomass at 22 d after application of four Italian ryegrass populations used in the paraquat dose-response experiment.\(^a\)

<table>
<thead>
<tr>
<th>Population</th>
<th>Regression parameters (±SE)(^b)</th>
<th>ED(_{50})(±SE)</th>
<th>ED(_{50}) R:S ratio(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(b)</td>
<td>(c)</td>
<td>(d)</td>
</tr>
<tr>
<td>Visual control rating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welcome (S1)</td>
<td>-2.32</td>
<td>1.40</td>
<td>98.21</td>
</tr>
<tr>
<td>White Castle (R1)</td>
<td>-0.55</td>
<td>-0.19</td>
<td>206.32</td>
</tr>
<tr>
<td>St. Gabriel (R2)</td>
<td>-0.93</td>
<td>1.23</td>
<td>112.04</td>
</tr>
<tr>
<td>Bunkie (R3)</td>
<td>-0.89</td>
<td>1.57</td>
<td>115.16</td>
</tr>
<tr>
<td>Dry weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Welcome (S1)</td>
<td>2.67</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
<td>White Castle (R1)</td>
<td>1.24</td>
<td>0.07</td>
<td>0.16</td>
</tr>
<tr>
<td>St. Gabriel (R2)</td>
<td>1.24</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>Bunkie (R3)</td>
<td>1.29</td>
<td>0.07</td>
<td>0.21</td>
</tr>
</tbody>
</table>

\(^a\) Abbreviation: ED\(_{50}\) is the herbicide rate expressed in g ha\(^{-1}\) that is required to provide 50% visual control or reduce dry weight by 50%.

\(^b\) The dose-response curve corresponding to parameters in this table can be found in Figure 1 for visual control ratings and Figure 2 for dry biomass. Regression parameters correspond to Equation 1 in the “Materials and Methods”. In this four-parameter logistic model, \(b\) is the slope of the cure, \(c\) is the lower limit for visually assessed control or biomass, \(d\) is maximum visually assessed control or biomass, and ED\(_{50}\) is the rate for 50% control or biomass relative to the nontreated check.

\(^c\) R:S (most resistant: most susceptible) rations were calculated by dividing the ED\(_{50}\) of the biotype of interest by the ED\(_{50}\) of biotype S1, the paraquat-susceptible biotype.
The resistant to sensitive ratio (R:S) is utilized as a resistance index and showed that the White Castle, St. Gabriel, and Bunkie populations were 200.4, 22.2, and 45.4-fold, respectively, less responsive to paraquat as compared to the Welcome population (Table 4). Tehranchian et al. (2018) reported that the MR1 population of Italian ryegrass was 20.5-fold less responsive to paraquat than the susceptible population. Certain populations of paraquat-resistant weeds such as horseweed and Philadelphia fleabane (*Erigeron philadelphicus*) are 450 and 250 times less responsive to paraquat as compared to their respective susceptible populations (Fuerst and Vaughn 1990).

Likewise, Italian ryegrass dry weight was modeled as a function of paraquat rate using a four-parameter log-logistic model. A lack of fit test was conducted and was not significant (P > 0.05). The ED$_{50}$ value required to reduce the dry weight by 50% was 44 g ha$^{-1}$ for the Welcome population, whereas the ED$_{50}$ for the White Castle, St. Gabriel, and Bunkie populations were 584, 188, and 643 g ha$^{-1}$, respectively (Figure 2 and Table 4). The resistant to sensitive ratio (R:S) indicates that it required 13.3, 4.3, and 14.3-fold more paraquat to reduce biomass, respectively, for the White Castle, St. Gabriel, and Bunkie populations as compared to the Welcome population.

With limited POST herbicide tools to manage Italian ryegrass in sugarcane, it is imperative that producers utilize PRE herbicides to aid in management. Utilization of residual herbicides such as $S$-metolachlor, metribuzin, diuron, and atrazine should be included as a complementary strategy for managing Italian ryegrass PRE in sugarcane (Orgeron 2021). Likewise, it is imperative to identify other POST control options and to develop IWM (Integrated Weed Management) strategies to aid in Italian ryegrass management in Louisiana.
sugarcane production due to the decreased sensitivity of some Italian ryegrass populations to paraquat.

Figure 2. Dose-response curves of Italian ryegrass biomass 22 d after treatment in response to increasing rates of paraquat. Plants were treated at 8- to 12- cm in height (two to five tillers). Dose-response curves were fitted using a four-parameter, log-logistic model in R software.
Chapter 3. Summary

Italian ryegrass is one of the more problematic winter grass weeds in sugarcane in Louisiana. The plant readily outcrosses with other ryegrass biotypes while being self-incompatible and reproduces by wind-distributed pollen. These aspects create an environment where herbicide resistance can rapidly increase in a population, simply because of the high degree of outcrossing in Italian ryegrass.

While in many cropping systems Italian ryegrass is commonly managed with glyphosate or ACCase herbicides, these herbicides are very damaging to sugarcane. Paraquat is the only labeled herbicide for use in sugarcane to manage Italian ryegrass POST. After reports of inadequate control of Italian ryegrass with paraquat in several Louisiana sugarcane fields, seeds were collected for evaluation and used to determine the sensitivity.

Seed from three populations of Italian ryegrass where inadequate control with paraquat was reported were collected from sugarcane fields in White Castle, LA (R1), St. Gabriel, LA (R2), and Bunkie, LA (R3). Additionally, Italian ryegrass seed were collected from a sugarcane field in Welcome, LA (S1) with no apparent paraquat control issues. Seed were germinated and grown to a five-tiller stage before being individually subjected to specific rates of paraquat, depending upon their classification. Populations suspected of reduced susceptibility were treated at paraquat rates of 0, 1/32 1/16, 1/8, 1/4, 1/2, 1, 2, 4, 8, and 16X, where 1x is the maximum field rate of 840 g ha⁻¹, whereas the susceptible populations were treated at rates of 0, 1/256, 1/128, 1/64, 1/32, 1/16, 1/8, 1/4, 1/2, 1, and 2X the maximum field rate. Treatments were applied using a DeVries Tracked Sprayer in ascending order to prevent rate cross-contamination. After
herbicide applications, visual injury ratings were recorded for each population, and biomass was harvested, dried, and weighed after the final visual injury rating.

Reduced paraquat susceptibility was observed within R populations confirming paraquat resistance. Using Welcome (S1) as the susceptible control population, the effective dose of paraquat to cause 50% visible injury (ED$_{50}$) was 77 g ha$^{-1}$, whereas the White Castle (R1), St. Gabriel (R2), and Bunkie (R3) populations required 15,482, 1,713, and 3,510 g ha$^{-1}$, respectively, for effective control. Accordingly, the three populations were 200.4, 22.2, and 45.4-fold less sensitive to paraquat than the Welcome (S1) population, respectively. The ED$_{50}$ value required to reduce the dry weight by 50% was 44 g ha$^{-1}$ for the Welcome population, whereas to reduced dry weight biomass for the White Castle (R1), St. Gabriel (R2), and Bunkie (R3) populations required 13.3, 4.3, and 14.3-fold more paraquat, respectively, as compared to the Welcome population (S1). These results confirm the presence of paraquat-resistant Italian ryegrass in Louisiana.

To reduce Italian ryegrass competition in sugarcane, especially in fields where paraquat-resistance is suspected, an effective solution is to incorporate PRE herbicides as an alternative to the failure of POST herbicide control options available. Herbicides providing residual control during PRE application, such as S-metolachlor and metribuzin, need to be used in IWM strategies prior to Italian ryegrass emergence. Furthermore, identifying alternative POST Italian ryegrass control options in sugarcane should be pursued.
References


Anonymous (2020a) Louisiana suggested chemical weed management guide. LSU AgCenter. https://www.lsuagcenter.com/~media/system/2/a/7/7/2a770792235b1e76eb1b7a6888fe1c8c/2020weedmgmtguidepdf.pdf. Accessed: August 29, 2020


Ghanizadeh H, Harrington KC, James TK (2015) Glyphosate resistant Lolium multiflorum and Lolium perenne populations from New Zealand are also resistant to glufosinate and amitrole. Crop Prot 78:1–4


http://dx.doi.org/10.1002/0471725315


Accessed:

January 17, 2020
Vita

Born on September 12, 1993, Alphonse Bienvenue Coco III is the son of Alphonse Coco II and Mary Perkins Coco. Raised in Tensas, Louisiana, he graduated from Tensas Academy in 2012 before enrolling at Louisiana Tech in the fall of 2012. Graduating from Louisiana Tech in May of 2016 with a bachelor’s degree in Biology, he began working for the LSU AgCenter at the Sugar Research Station in Saint Gabriel, where he eventually attained the position of Research Associate. In 2018, Alphonse began taking classes before enrolling in the fall of 2019 in the School of Plant, Environmental and Soil Sciences under the direction of Dr. Albert Orgeron. He will graduate in the fall of 2022 with his Master’s degree in weed science.