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Examination of Pre-emergence Control of Johnsongrass in Sugarcane Seedlings

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EXAMINATION OF PRE-EMERGENCE CONTROL OF JOHNSONGRASS IN SUGARCANE SEEDLINGS

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Abstract

Sugarcane is susceptible to many diseases and insect pests; therefore, to maintain high sugar yield new varieties must be developed. Producing new varieties contributes to the overall crop success through enhanced yield, insect and/or disease resistance, cold tolerance, and ratooning ability. However, unlike conventional sugarcane, which is vegetatively propagated, new sugarcane varieties are produced from true seed. These seedlings are more susceptible to herbicidal injury and weed competition than conventional sugarcane. For sugarcane seedlings to succeed, weeds must be controlled therefore, the most effective herbicide program with regards to seedling safety must be implemented. The objectives of this research were to determine the safety of several preemergence (PRE) herbicides on sugarcane seedling crosses and to evaluate their efficacy in controlling seedling johnsongrass. Field studies were conducted at the LSU AgCenter's Sugar Research Station in 2019 and 2020 to evaluate crop injury, seedling mortality, and yield of ten sugarcane crosses against seven PRE herbicide treatments applied directly after seedlings were transplanted into the field. Of the seven herbicide treatments evaluated, three of the treatments contained the active ingredient *S*-metolachlor which was labeled for use in sugarcane production in 2018. Results revealed that metribuzin (1.68 kg ha^{-1}) was the only treatment that significantly increased sugarcane seedling mortality at 90 days after the transplanting procedure. Supplementary experiments were conducted in 2020 at the LSU AgCenter's Sugar Research Station to test the efficacy of these treatments in controlling seedling johnsongrass. Plots were overseeded with johnsongrass seed and were shallowly tilled prior to herbicide application. Johnsongrass emergence was counted 7, 14, and 28 days after treatment (DAT), and johnsongrass dry weight was measured 28 DAT. Results showed that pendimethalin and metribuzin provided the best control of johnsongrass seedlings, and control with *S*-

metolachlor was not as sufficient. Based on the results of these studies, pendimethalin at 2.32 kg ha^{-1} is a sound option for controlling seedling johnsongrass without compromising the survival of newly established sugarcane.

Chapter 1. Introduction

Sugarcane (*Saccharum spp.*) is a large perennial grass grown in tropical and semitropical climates. The above ground structure of sugarcane consists of a photosynthetic canopy of leaves and juicy fibrous stalks ranging from 1 to 3 m in height and 2 to 5 cm in diameter (Lingle 1999). Sugarcane is a C_4 crop known for its ratooning ability and its production of sucrose. Sucrose is primarily refined into table sugar for human consumption, but it can also be fermented to create ethanol and used as a fuel or other industrial purposes. Current commercial varieties in Louisiana can produce anywhere from 5.7 to 10.9 metric tons of sugar per hectare (ha) depending on the variety and the age of the crop (Gravois 2021).

Sugarcane is one of the largest cultivated crops in the world with over 26 million ha of land devoted to its production and over 190 billion tons produced globally in 2018. Sugarcane is grown in over 90 different countries including Brazil, India, China, Australia, and the United States (FAO 2019). In 2020 there was approximately 370,000 ha of commercial sugarcane production in the United States. Domestic sugarcane production occurs in Louisiana with 190,000 ha, Florida with 166,000 ha, and Texas with 13,600 ha (USDA 2020) Sugarcane also provides useful by-products such as molasses and bagasse. Molasses is often used as an additive in animal feeds and can also be used in the production of liquors such as rum. Bagasse is burned to produce steam energy, which is often used to run sugar mills, and can be used as a fuel source to cogenerate electricity (Pollack 1995; Purchase 1995).

History of Sugarcane

Sugarcane was originally domesticated in New Guinea around 8000 BC (Godshall and Legendre 2003). From there sugarcane cultivation spread through Eurasia and into India. When colonization of Louisiana began sugarcane was one of the first major crops introduced.

Sugarcane has undergone many changes since it was originally domesticated. The varieties that are grown today grow far larger and produce much more sugar than the original introductions brought to the new world by Columbus in 1493. Sugarcane production in the new world began on the Caribbean islands where it quickly gained popularity. Large amounts of liquid sugar were shipped from the Caribbeans to France to be refined creating a successful trade market (Mullenix 2002). The first variety to be cultivated in Louisiana was an Indian cultivar known as “Creole”. However, this variety was quickly replaced by “noble” sugarcane varieties which were discovered in the western Pacific and were shipped to the Caribbeans in 1789 (Patterson 2013). These varieties were produced until Sereh disease began to impact their yield forcing the establishment of the first breeding programs in Barbados and Java in 1888 (Heinz 1987). These breeding programs developed hybrid varieties of sugarcane by crossing local commercial sugarcane varieties with wild ancestral clones.

Sugarcane was originally introduced to America by Pierre Le Moyne D'Iberville who planted varieties from St. Domingue along the lower Mississippi river in the early 1700’s (Mullenix 2002). However, it was not until the early 1750’s that a successful sugarcane crop was raised by Jesuit missionaries in what is now downtown New Orleans. Afterwards many other plantations also began producing sugarcane. Further success came when cane growers from the Caribbeans were forced to relocate due to a violent revolt against plantation owners. In 1795, Etienne de’Bore was credited with making the first granulated sugar in New Orleans. His sugarcane crop produced over 50 tons of sugar. This success inspired more farmers to join their endeavor, eventually establishing Louisiana as a major producer of sugarcane in North America. Cane production in Louisiana continued to expand and in 1904 growers were able to produce almost 400,000 tons of sugarcane. However, by 1926 the yield had dropped to 50,000 tons, largely due to impacts from sugarcane mosaic disease (SCMV).

SCMV destroys the chlorophyll of infected plants, which can significantly impact its photosynthetic capacity (Bagyalakshmi et al. 2019). A reduction of photosynthesis can impact root and shoot growth as well as tillering, thus lowering yield due to smaller and fewer stalks. SCMV can also reduce sucrose content as well as crystallization rate and has shown it can reduce yield up to 80% (Grisham 2011; He and Li 2006). To save the sugarcane industry the United States Department of Agriculture (USDA) obtained three disease resistant varieties from the experimental station in Java. POJ 36, POJ 213, and POJ 234 which exhibited resistance to SCMV were imported to the US providing an alternative to the existing susceptible varieties. These new varieties facilitated the survival of the industry until the USDA breeding programs could supply new varieties (Mullenix 2002). Two USDA breeding programs were formed, one located in Canal Point Florida in 1919, and the other located in Houma, Louisiana in 1923.

Developing a Sugarcane Variety in Louisiana

Variety development is a complex procedure. This process begins by choosing different varieties, based on desirable morphologic and genetic characteristics, which will be used as parents the following year. These varieties are grown in a greenhouse before being transferred to photoperiod manipulation bays. Sugarcane does not flower under natural conditions in Louisiana. Temperatures below 18.3^{o} C prevent flowering during the tassel initiation phase. This temperature can also cause low pollen fertility in existing flowers (Chilton and Paliatseas 1956; Thompson 1984). Sugarcane is a short-day plant, and it uses a photoreceptor protein to detect changes in day length which it then takes as a signal to flower. There are three different responses to day length, there are short day, long day, and day neutral plants. Short day plants flower when the night length exceeds their critical photoperiod while long day plants flower when night length falls below their critical photoperiod, day neutral plants do not flower according to changes in day length (Mauseth 2003). Sugarcane's flowering response to short days was first discovered in 1940 by South African researchers who developed a system to artificially induce sugarcane flowering. They achieved this by manipulating the number of hours of exposure to darkness. In 1956 the Louisiana Agricultural Experiment Station under the direction of Dr. Chilton began using this system to initiate sugarcane flowering in Louisiana (Gravois 2019).

Sugarcane flowering occurs when an open branched panicle called a tassel is formed. The tassel consists of thousands of tiny flowers, each flower can produce one seed. Once one of the sugarcane stalks forms a tassel that stalk is placed in the crossing house and the flower is analyzed under a microscope to determine the amount of pollen produced for each flower. Varieties with abundant pollen are considered male clones, and varieties with little to no pollen are considered female clones. The breeder attempts to make the best crosses by pollinating female flowers with male flowers. Each variety reacts differently to photoperiod manipulation therefore flowering occurs at different times. Crossing selection is determined by flower availability with reference to an additive genetic model (Gravois et al. 1991). Once the flowers have been pollinated the true seed is collected.

Louisiana’s climate and the slow rate at which seedlings grow make the timing of their transplant into the field critical. To achieve full canopy closure, the seedlings should be transplanted as early as possible. However, freezing temperatures in early spring must be avoided. Because of this the timing of seedling production in Louisiana must follow a strict timeline. In January, the true seed is planted in trays which have been placed on a warmed sand bed within a greenhouse and then filled with plant media. A light layer of the media is then applied to cover the seed which is then watered regularly. The seed typically germinates seven days following planting. Each seedling that germinates is a genetically unique individual, with the potential to become a new variety. Seedlings are allowed to grow one to two months until they are sturdy enough to be transplanted into individual cells within a 128-cell tray. Afterwards they are placed in a separate greenhouse where they are allowed to grow for two more months. Once the seedlings have established a substantial (or healthy) root system they are planted in a field in April. Seedlings are planted 40 cm apart on 1.8 m row centers. These seedlings will not be assessed for continuation in the program until the fall of the following year. For example, seedlings planted in the spring of 2021 will not be assessed until the fall of 2022, during the first ratoon crop. During this time seedlings will be allowed to grow until they are harvested in December 2021.

New growth will then emerge from the root structure which was established from the seedling the previous year, this is called ratooning and it is an important trait in sugarcane. A seedling's ratooning ability can only be observed after the crop has been harvested, which is the reason for this delayed assessment. Around September, selection is practiced among these seedlings. Plants showing ideal traits such as adequate stalk diameter, stalk height, stalk population, and good ratooning will be advanced to the next selection stage (Bischoff and Gravois 2002). Roughly 60,000 to 90,000 seedlings are planted each year at the LSU AgCenter's Sugar Research Station in St. Gabriel, Louisiana, from this only 2,000 to 3,000 are selected to move to the next stage of the program (first line trials).

There are many reasons seedlings may not be considered for continuation in the program: poor growth, presence of a disease such as smut, tube (hollow core in the center of the stalk) and pith (dead parenchyma cells) formation in the stalk, lodging or open growth traits. These are all reasons for seedlings to be eliminated from the program.

However, elimination due to a seedling's inability to express their full potential caused by resource competition from heavy weed pressure is not acceptable. At this stage seedlings only have one chance to be selected for continuation in the program; therefore, it is crucial to maintain a weed-free growing environment in seedling fields. This is difficult because of plant spacing and general lack of vigor in this first stage of the breeding program. After being selected these varieties continue through the next stages of the variety development program. These stages involve planting the varieties in larger plots and eventually in different locations throughout Louisiana, this process takes about 12 years to complete. Upon completion of the process, varieties that excel in yield and have desirable characteristics are released to Louisiana producers for commercial production.

Seedling Sugarcane versus Production Sugarcane

While sugarcane plants can arise from true seed which are produced during hybridization, commercial sugarcane varieties are produced via vegetative propagation. Vegetative propagation depends on a vegetative part of a plant such as the stem to act as the parent plant (Britannica 2017). Sugarcane stalks are made up of multiple segments called joints, each joint contains a node and an internode. The node is the point at which the leaf attaches to the stalk, each node contains a bud and root primordia. When planted, a primary shoot will develop from the nodal bud, then secondary shoots called tillers emerge from the primary shoot, an initial root structure also forms at each node (Sandhu et al. 2019). The stalks that emerge following planting are genetically identical to the seed cane utilized for propagation.

This method of reproduction has both advantages and disadvantages. Sugarcane stalks have large reserves of carbohydrates that allow for faster growth of the developing roots and shoots. Sugarcane true seed (derived from the crosses of flowers of two parents) are small and do not have this reserve of carbohydrates therefore their growth and development occur at a much slower rate. This slower growth rate is an important factor when considering weed control in sugarcane. In good growing conditions sugarcane will form a canopy which provides natural protection from weeds by intercepting the light required for weed germination (Bittencourt 2009). A complete canopy takes longer to form in seedling fields due to this slower growth, plant spacing and other factors, all of which can provide more opportunities for weed populations to become established.

If seedlings are spaced too closely, they will not be able to grow beyond a certain capacity without causing inter-plant competition among neighboring seedlings. In order to provide each seedling with the opportunity to fully express its potential, each plant is spaced 40 cm apart. This separation reduces competition for light, water, and nutrients. As sugarcane grows it produces new shoots called tillers, tillering expands outwards from the initial crown. If a cross has good tillering and the seedlings are planted close together it can be difficult to distinguish between seedlings during the selection stage. However, if a cross does not have good tillering or good growth in general this spacing can provide an opportunity for weed growth.

Each sugarcane seedling is genetically unique. Because of this, growth is not consistent between seedlings. When a cross does not perform well canopy closure can take longer or may not occur at all. Poor ratooning ability and poor cold tolerance are genetic traits which can affect canopy closure in the first ratoon crop (Bischoff and Gravois 2002). In some cases, entire crosses may have little to no growth in the first ratoon crop which provides further opportunities for weed populations to become established (M. Pontiff, personal communication, October 15, 2021). Sugarcane is a tropical crop, with the base temperature for tillering to occur is 16^{o}C but the ideal temperature is 30^{o}C (Gascho et al. 1973). In Louisiana temperatures are only in this ideal growth range from late April until early November, growth is slower outside of these months which can contribute to a slower canopy closure. An additional factor that affects canopy closure in Louisiana is the practice of planting on a 1.8 m row spacing as opposed to a 1.5 m row spacing used in many other sugarcane growing regions.

The Importance of New Varieties

Commercial sugarcane is vegetatively propagated to begin a new crop cycle. While this produces a true clone of the mother plant, there are some disadvantages. One disadvantage is the ease with which systemic pathogens can be transferred from seed cane to new sugarcane plants. This gradual accumulation of bacteria, viruses, and fungi can lead to a loss of vigor in varieties and can lead to varietal degeneration. As hectarage of varieties are increased following commercial release, the number of diseases and environmental stresses are likewise increased. These environmental stresses include extreme drought or flooding, inadequate soil fertility, increased insect infestations and increased disease levels. A leading variety, which makes up the majority of the commercial acreage, is exposed to a higher level of disease and therefore could be more susceptible to varietal degeneration. Some sugarcane diseases, such as brown rust (*Puccinia melanocephala*), can adapt as new varieties dominate the industry. While there are some methods to slow this varietal degeneration, such as clean seed programs and strategic varietal selection, an eventual loss of vigor generally occurs over time (Viswanathan 2015).

Because sugarcane is susceptible to varietal degeneration new varieties must be produced to replace those varieties which can no longer produce adequate yields. The sugarcane industry is aware of this demand and in turn variety development programs were formed. In Louisiana, two programs exist. One is located at the Sugar Research Station in St. Gabriel, LA with the Louisiana State University Agricultural Center, and the other is located at the Sugar Research Unit in Houma, LA. with the USDA Agriculture Research Service. These organizations work together in conjunction with the American Sugar Cane League to develop new varieties for the Louisiana industry. The main goal in producing new varieties is to improve sugar yield and disease and insect resistance.

Problems in Sugarcane Production

Sugarcane is vulnerable to a variety of pest problems including insects, diseases, and weeds. Weed competition in the early growing season is of particular concern because of seedling sugarcanes slow initial growth and development (Sandhu et al. 2019). If weed populations become established, they will reduce sugar yield by competing for limited resources including nutrients, light, and water (Ross and Fillols 2017). Additionally, an early infestation of weeds can have a negative effect on tillering in sugarcane, lowering the amount of millable stalks and reducing cane yield (Odero et al. 2016). Therefore, the timing of the removal of these weed populations is crucial and is referred to as the critical period for weed control (CPWC). The CPWC is the time between the critical time of weed removal (CTWR) and the critical weed free period (CWFP). CTWR is the amount of early weed interference a crop can tolerate before incurring permanent losses and CWFP is the period of time after planting that fields must remain weed free to prevent major losses. The CPWC varies between varieties, one study found that the CPWR of fall panicum ranged from 4.6 to 8.1 weeks after emergence to prevent a 5 to 10% yield loss (Odero et al. 2016).

Some methods of controlling weed populations include cultural, mechanical, and chemical control. Cultural weed control practices such as cover cropping, crop rotation, canopy development and mulching from leaf residue can reduce weed populations (Archana 2020). Mechanical cultivation controls existing weed populations by uprooting or severing their roots. This method only controls emerged weeds that are in the row middle and can be conducive to additional germination of other weeds by bringing them to a different point in the soil profile. Chemical control uses the application of herbicides in either a preemergence (PRE) or postemergence (POST) application. PRE herbicides are applied after planting and before weeds or sugarcane have emerged from the ground while POST herbicides are applied after weed emergence (Rott et al. 2018).

Chemical Control

There are many considerations to achieve proper weed management of sugarcane. The first aspect which must be considered is the array of weeds which are present in sugarcane fields or within the seedbank and thus will determine the appropriate weed management strategy. Management strategies must consider current production (fallow vs in-production), application timing (burndown, spring, and layby), herbicide spectrum and problematic-yield limiting weeds. Sugarcane fields are often in production for 3 to 4 years or longer, during this time weed populations can become compounded. Because of this, fallow land preparation can be one of the most important strategies in a weed control program. If done properly a grower can reduce the amount of weed seeds and rhizomes which in turn reduces weed pressure in the plant cane crop. Fallow land preparation implements a combination of deep tillage as well as applications of glyphosate and soil applied herbicides (Griffin et al. 2001; Orgeron 2019).

Planting of sugarcane in Louisiana occurs during late July through early October allowing time for germination and plant formation before winter freezes kill the parts of the plant above the soil surface. Growth resumes in the spring as temperatures begin to rise and dormancy ends. The initial period of regrowth is also the CPWC for sugarcane. To ensure sugarcane has a competitive advantage at this time weed populations must be properly managed. According to the LSU AgCenter's Chemical Weed Management Guide the best way to achieve weed control is through an application of PRE herbicides immediately after planting. A follow up application should also be applied 60 days later to extend the control of summer weeds and provide some residual control of winter weeds. Some examples of these PRE herbicide applications in sugarcane would be clomazone (Command® 3ME, FMC) at 1451 g ha^{-1} plus diuron (Direx® 4L, Adama Essentials) at 2849 g ha^{-1} at planting followed by an application of metribuzin (Sencor® DF, Bayer) at 1260 g ha^{-1} 60 days later; hexazinone (Velpar® L, Bayer) and trifluralin (Treflan® 4L, Dow Agriscience) can also be used in these formulations (LSU AgCenter 2022).

Sugarcane is dormant during the winter months. During this time winter annuals can begin germinating these include Italian ryegrass (*Lolium multiflorum*), bluegrass (*Poa annua)*, rescuegrass (*Bromus catharticus*), timothy canarygrass (*Phalaris angusta*) and an assortment of broadleaves (Griffin et al. 2001; Orgeron 2018). If left untreated these weeds can interfere with sugarcanes growth as it emerges from dormancy. If these weeds are not controlled by the residual effects from an early application a POST application is necessary. Most broadleaves can be controlled with an application of 2,4-D at 552 to 1,656 g ha^{-1} or a premix of 2,4-D and dicamba (Weedmaster®, Nufarm), at 562 to 1,125 g ha^{-1}. There are some broadleaves however which cannot be controlled with 2,4-D and dicamba. In 2010, a new broadleaf weed species was observed in a commercial sugarcane field after 2,4-D and dicamba provided poor control. This weed began to quickly spread throughout the Louisiana sugarcane industry and was identified as divine nightshade (*Solanum nigrescens*) (Orgeron et al. 2018). Control of annual winter grasses can be achieved through an application of paraquat (Gramoxone® SL 2.0, Syngenta) at 702 g ha^{-1}. In order to provide a weed-free environment for sugarcane emerging in the spring herbicide programs should be implemented in February or March. PRE herbicide should be applied after cultivation of the row sides and middle. This application can be broadcasted; however, in most cases, it is applied in a band across the top of the cane to reduce the cost per acre (Griffin et al. 2001). Common PRE formulations that are used include pendimethalin (Prowl® H2O, BASF) at 2,172 to 3,258 g ha^{-1} and metribuzin at 1,681 to 3,363 g ha^{-1} (LSU AgCenter 2022).

Grasses like johnsongrass (*Sorghum halepense)* and itchgrass (*Rottboellia cochinchinensis*) which survived the PRE herbicide can be controlled with a POST application of asulam (Asulox®, UPI) at 3.75 kg ha^{-1}. An additional application of asulam may be necessary after eight weeks to control regrowth (LSU AgCenter 2022). When applying asulam it is important that the soil is not disturbed seven days before or after the application and a rain-free period of at least 20 hours after application is desirable to optimize control. Asulam can cause phytotoxicity and stunting of growth when sugarcane is stressed from drought or when temperatures are above 30^{o}C. Because of this it is recommended to not apply asulam after mid-May to early June (Griffin et al. 2001).

A final layby application of PRE herbicides is made prior to canopy closure to aid in controlling morningglory species and other problematic grasses. The layby application should be made following the final cultivation of the row middle and should be directed underneath the crop canopy. In some cases, vines like morningglory species will grow after this layby application. Vines can climb sugarcane and form a dense canopy over the cane which can have a negative impact on cane growth and can cause difficulties when harvested. An aerial application of 2,4-D or atrazine can be applied for POST control of these vines.

New Chemical

In 2018 a new chemical, Lumax® EZ (Syngenta) was registered for use in sugarcane. Lumax EZ is a premixed herbicide which contains three active ingredients (ais) mesotrione, atrazine, and *S*-metolachlor. This is the first PRE that has been approved for use in sugarcane since pendimethalin in 1993. Additionally, three herbicides which solely contain *S*-metolachlor were labeled for sugarcane use in 2020, namely StrelluiS™ II (Atticus, LLC), Visor® S-Moc (Innvictis) and, Visor® S-Moc II (Innvictis). *S*-metolachlor is a new active ingredient to the United States sugarcane industry and it is the only group 15 herbicide labeled for use in domestic sugarcane production. Group 15 herbicides are long chain fatty acid inhibitors, that target a plants ability to synthesize long chain fatty acids which are necessary for plants shoot development. Metolachlor was first created in 1976, and contained four isomers: two R isomers and two S isomers. It was later discovered that the S isomers provided the most herbicidal activity (Moser et al. 1983). In the 1980's, a method was developed to enrich the isometric ratio of metolachlor to favor the *S* isomer. This formulation is more environmentally safe because it allows for a lower use rate while still providing similar control of grasses and broadleaves. *S*-metolachlor is used as a PRE in many crops including corn *(Zea mays)*, soybean *(Glycine max)*, and sugar beet *(Beta vulgaris*) (O'Connell et al. 1998). Lumax EZ can be an effective tool in sugarcane production, it provides PRE control of many weeds and contains three different modes of action (MOA) which could help prevent herbicide resistant weeds from developing.

Johnsongrass

Johnsongrass (*Sorghum halepense* L. Pers.) is the main weed concern in sugarcane seedling fields on the LSU AgCenter's Sugar Research Station. Johnsongrass is a noxious weed with a worldwide distribution that negatively affects the yield of many field crops; some examples include corn, soybean, sugarcane, and cotton (*Gossypium hirsutum*). Johnsongrass can impact yield either by competing for resources, by acting as a host for numerous pathogen species, or by exuding allelopathic toxins from its roots (Parsons and Cuthbertson 1992; Vasilakoglou et al. 2005). In sugarcane, johnsongrass has shown to cause up to an 84% reduction in yield and can cause some fields to be removed from production after only one year (Arevalo et al. 1977; Millhollon 1970). Johnsongrass can grow up to 2 m in height and can be reproduced from a seed or from a rhizomatous root structure. One plant can produce up to 28,000 seeds in a year and rhizomes can grow one meter per day (Horrowitz 1973). Johnsongrass seed can remain dormant and viable up to 10 years which allows for continual reinfestation and a large build up in the weed seedbank (Huang and Hsiao 1987; Johnson et al. 1997). Rhizomes can form 18 days after seedling emergence and can produce new plants even when cut away from the host plant (Horowitz 1972; McWhorter 1961b).

Some of the most noxious weeds sugarcane growers deal with are grasses. This is largely due to the availability of selective herbicides which only target broadleaf plants. Sugarcane is a grass; therefore, these selective herbicides can be applied with no negative affect to the crop. However, when dealing with grass weeds many of the mode of actions (MOA) which control these grasses would also have a negative impact on sugarcane. One example of this is glyphosate, which is recommended to control rhizome johnsongrass during the fallow period. There are no sugarcane varieties which are glyphosate tolerant (Roundup Ready®) in the United

States, so if applied to sugarcane, it would have an extremely detrimental effect on growth. However, there are a limited number of herbicides which provide POST control over grass weeds like johnsongrass in crop; namely asulam and trifloxysulfuron-sodium (Envoke®, Syngenta).

The most effective method of controlling johnsongrass is through prevention, achieved through proper fallow land preparation using both cultivation and chemical control. Cultivation can break up the rhizomatic structures however, because these structures can still produce new plants after being cut, follow up chemical control is still required (Griffin et al. 2001). Following planting, applications of PRE herbicides should be applied to prevent new johnsongrass populations from forming. According to the LSU AgCenter's Chemical Weed Management Guide (2022) some chemicals which provide PRE control of johnsongrass include clomazone, metribuzin, pendimethalin, trifluralin, and hexazinone. However, these only provide control over johnsongrass formed from seed not rhizomes. Asulam can provide POST control of johnsongrass plants which are 30 to 45 cm tall. If the plant gets much larger than 45 cm or begins to develop seed, the degree with which it can be controlled is greatly reduced (McWhorter 1961a).

Little research has been conducted on sugarcane seedlings to evaluate their potential increased sensitivity to herbicides. However, anecdotal observations suggest that seedlings are more susceptible to herbicidal injury following the transplanting process. Therefore, any herbicides which cause phytotoxicity in sugarcane could have a more detrimental effect on sugarcane seedling growth and survival. The combination of increased weed pressure and herbicidal sensitivity creates a unique challenge when discussing weed management in sugarcane seedlings. Further research must be done to determine which herbicides are safe for use on sugarcane seedlings. The objectives of this project were:

1. to determine if the PRE herbicides commonly used in conventional sugarcane production are safe to use on sugarcane seedlings when applied directly after being transplanted into the field.
2. to determine the efficacy of commonly used PRE herbicides in controlling seedling johnsongrass.

Chapter 2. Preemergence Herbicide Effects on Sugarcane Seedlings

Introduction

Sugarcane (*Saccharum spp.*) is the highest valued row crop in Louisiana producing 15.5 million tons in 2020 (USDA 2020). Variety development plays a major role in the continued success of the sugarcane industry. The method with which commercial sugarcane is reproduced makes it susceptible to an accumulation of diseases. This can lead to a loss of vigor and eventually a decline in yield which is called varietal degeneration (Viswanthan 2015). New varieties produced in a variety development program replace older varieties that no longer meet industry expectations for sugar yield. These new varieties are produced by crossing two existing varieties, the resulting true seed is genetically unique. This seed is grown in a greenhouse until they are large enough to be transplanted into a field. The seedlings are then observed in a series of stages which gradually increase in size and location. Any varieties that emerge from this program are commercial varieties (Bischoff and Gravois 2002).

Sugarcane seedlings are a vital component of the variety development program. However, there are some differences between seedling production and conventionally grown cane which can affect the way in which they are managed. Some of the differences include inconsistent and slower growth, and an increased sensitivity to herbicides. Sugarcane can develop a full canopy which can provide excellent natural weed control (Bittencourt 2009). When growth is slower as is with sugarcane seedlings the canopy takes longer to develop and therefore provides more opportunities for weed populations to become established. While little research has been conducted on the subject, anecdotal observations suggest that sugarcane seedlings may be more susceptible to herbicidal injury. This increased susceptibility can prevent the use of some herbicides which may be better at controlling certain weed populations. One example of this is asulam (Asulox®, UPI), which is a selective POST herbicide that targets grasses and is an effective tool for controlling rhizome johnsongrass. However, asulam is known to cause phytotoxicity to sugarcane when applied during periods of drought or when high temperature conditions exist in June. Because of the potential increased sensitivity to herbicidal injury among sugarcane seedlings, asulam is rarely used in seedling fields thus reducing the options for effective POST control of johnsongrass.

To provide the best growing environment for sugarcane seedlings, weed control must be achieved immediately following transplanting. The critical period for weed control in sugarcane is the time before and during tillering (Odero et al. 2016). Tillering is the physiological process in which continuous branching of underground nodes result in the production of additional sugarcane shoots. Tillering is an important process as it increases the amount of millable stalks thus increasing crop yield (Vasantha et al. 2012). If weed populations are not controlled at this time, they can prevent tillering by shading out emerging shoots (Odero et al. 2016). Early weed control is typically achieved through the use of preemergence (PRE) herbicides. PRE herbicides provide residual weed control which prevents the establishment of weeds for several weeks. If weed populations are left unchecked, they can severely impact the development of seedling growth which can, in return, inhibit their continuation in the program. This result is undesirable because a seedling which could become the next leading variety may be eliminated from the program before it has a chance to fully express its potential.

Lumax® EZ was approved for use in sugarcane in 2018, and contains three active ingredients (ai's) mesotrione, atrazine, and *S*-metolachlor. In 2020, StrelluiS™ II, Visor® S-Moc and, Visor® S-Moc II were also labeled for sugarcane use and contain the ai *S*-metolachlor. *S*-metolachlor is a group 15 herbicide which is a new mode of action (MOA) for the United States' sugarcane industry. Group 15 herbicides are long chain fatty acid inhibitors (LCFAI) they prevent the production of fatty acids which are necessary for plant growth (Price et al. 2015). *S*-metolachlor is used for PRE control of some broadleaf weeds and annual grasses in many crops including corn *(Zea mays)*, soybean *(Glycine max)*, and sugar beet *(Beta vulgaris*) (O'Connell et al. 1998). Lumax EZ can be an effective tool in sugarcane production, as it provides PRE control of numerous broadleaf weeds and annual grasses and uses multiple MOAs, which helps prevent the development of herbicide resistance.

The use of PRE herbicides can reduce undesired weed populations thus providing a more favorable growing environment for sugarcane seedlings. However, anecdotal observations suggest sugarcane seedlings may be more susceptible to herbicidal injury following the transplanting procedure. Little research has been conducted to determine the impacts that these PRE herbicides can have on sugarcane seedlings. For this project a focus was placed on *S*-metolachlor due to its recent introduction to the sugarcane industry. Therefore, the objective of this study was to determine if the PRE herbicides commonly used in conventional sugarcane production are safe to use on sugarcane seedlings when applied directly after being transplanted into the field.

Materials and Methods

Study Site and Experimental Design

A field experiment was conducted during 2019 and 2020 to determine the impact of seven PRE herbicide treatments on sugarcane seedling families at the Sugar Research Station at St. Gabriel, Louisiana (30.267221 Lat –91.106431 Lon). Sugarcane seedlings from ten families with diverse lineage were used for this experiment. True seed from each family was planted in separate 35 by 50-cm trays filled with growing media (Pro-Mix FLX Premier Horticulture Inc., Quakertown, PA) on January 22, 2019, and January 23, 2020. Trays were placed on heated sand beds within a greenhouse and were maintained at 35°C.

Planting was achieved by spreading the true seed across the entire tray, then a light layer of growing media was used to cover the seed. Trays were watered and a sterilized plastic lid was placed over each tray. The heated sand bed and plastic lid act as a greenhouse within a greenhouse, providing the proper environment for germination at this time of year. Following planting, trays were watered daily. Additional maintenance included the periodic trimming of seedlings to a height of 10 cm. Trimming of seedlings reduces competition between seedlings and provides the opportunity for all possible seed to germinate. The seedlings were given three to four weeks to germinate and become rigid enough to transfer to individual cells (3.8 cm by 3.8 cm) in a 128-cell polystyrene tray (Speedling Transplant Trays TR128A, Speedling, Ruskin, FL).

Once transferred the seedlings were placed in a greenhouse where they were watered daily. Seedlings were trimmed to a height of 10 cm weekly using an electric hedge clipper to reduce competition and help strengthen the stalk. Following transplanting, a 20-20-20 N-P-K fertilizer (Peters' Professional 20-20-20 General Purpose Fertilizer, The Scotts Company, Marysville, OH) was applied weekly, 11.3 kg of this mixture was added to 113.6 L of water to form a solution of 1 part fertilizer to 100 parts of water. The seedlings were allowed to grow six weeks to develop a root structure and gain vigor before being transplanted.

Sugarcane seedlings were transplanted into a commerce silty loam (*fine-silty, mixed, nonacid, thermic Aeric Fluvaquent*) soil; pH of 7.3 and 1.3% organic matter using a carousel 2-row planter (Mechanical Transplanter Company, Holland MI) onto 1.8 m raised beds on April 30, 2019, and April 21, 2020.

The experiment was conducted as a split-plot design and treatments were replicated four times. Sugarcane seedling family was considered the whole-plot treatment and consisted of 112 sugarcane seedlings from ten diverse sugarcane cross families (Table 2.1) and were planted 45 cm apart on two adjacent rows.

Table 2.1. Sugarcane seedling family cross and sugarcane parentage used to conduct herbicide tolerance evaluations at the LSU AgCenter's Sugar Research Station in 2019 and 2020.

Family Cross	Cross Number	Female Parent	Male Parent
7	CP17-0513	HoCP 09-804	L 12-201
138	CP18-0194	L 09-112	L 12-201
162	CP18-0285	L 14-282	Ho 12-630
166	XL 18-166	L 14-273	L 09-099
170	XL 18-170	L 14-275	L 12-202
192	XL 18-192	Ho 11-9406	18P12
231	CP18-0512	L 15-317	L 12-201
242	CP18-0647	L 14-282	HoL 15-993
288	CP18-1040	Ho11-573	Ho 12-630
289	CP18-1041	Ho13-708	Ho 12-630

Herbicide treatment was considered the sub-plot and consisted of seven different PRE herbicide treatments as well as an untreated check. Herbicide treatments were randomly assigned to whole-plots and were applied to a series of 14 consecutive seedlings immediately after planting. Herbicide treatments were applied using a handheld CO_2 pressurized backpack sprayer equipped with AIXR 11002 flat fan nozzles (TeeJet® Technologies, Spraying Systems Co., Wheaton, IL 60187) calibrated to deliver 140 L ha^{-1}. Herbicide treatments were:

1: *s*-metolachlor (Medal II EC, 1.05 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409),

2: *s*-metolachlor (Medal II EC, 2.10 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409), 3: *s*-metolachlor + atrazine + mesotrione (Lumax EZ, 1.55 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409) at,

4: mesotrione (Callisto 0.105 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409) + atrazine (Atrazine 4L, 1.68 kg ha^{-1}; Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632),

5: metribuzin (TriCor 75DF 1.68 kg ha^{-1}; UPL NA Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406),

6: pendimethalin (Satellite Flex, 0.93 kg ha^{-1}; UPL NA Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406) + atrazine (Atrazine 4L, 2.24 ha^{-1} ; Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632),

7: pendimethalin (Satellite Flex, 2.32 kg ha^{-1}; UPL NA Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406) + atrazine (Atrazine 4L, 2.24 kg ha^{-1} ; Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632),

8: untreated check.

Weeds were hand-removed from all plots for the duration of the study in order fair evaluate the impact of the herbicide treatments.

Data Collection

Evaluations on herbicidal injury, seedling survival, and plot biomass were used to determine the antagonistic or neutral effects of each treatment on sugarcane seedlings. The process of being transplanted can cause stress to seedlings. Signs of stress are exhibited as wilting or scorching of leaves which can be similar to herbicide injury. Because of this, observations of injury were made by comparing each treatment to the untreated check within the same whole-plot. Sugarcane seedling injury ratings were made 7 days after treatment (DAT) and were recorded on a percentage bases using a scale from 0 to 100%. Where 0% shows no distinguishable injury when compared to the check and 100% being complete sub-plot death. Sugarcane seedling survival was recorded at 90 DAT. Sugarcane was harvested using a commercial sugarcane combine harvester in December of the year of planting, and sugarcane was loaded into a wagon equipped with loadcells to determine sub-plot biomass.

Statistical Analysis

The MIXED procedure in SAS (SAS 9.4 Institute, Cary, NC) was used to perform a generalized linear analysis of variance for sugarcane seedling injury, mortality and biomass. The UNIVARIATE procedure was used to test for normality. Means were separated using the Tukey range test ($P<0.05$), and letters were assigned to display significant differences. Sugarcane seedling crosses and herbicide treatment and their interaction were considered as fixed effects whereas replication, year, and replication nested within year were considered random effects.

Results and Discussion

The ANOVA analysis showed no significant interactions among sugarcane cross and herbicide treatment; hence, only main effects will be discussed (Table 2.2). Sugarcane cross had a significant ($P\leq 0.05$) effect on sugarcane biomass and seedling survival (Table 2.2). Biomass and seedling survival were averaged across herbicide treatment, and biomass ranged from 14.6 to 19.8 Mg ha^{-1} and sugarcane seedling survival ranged from 81 to 93% (Table 2.3). This is an expected outcome which demonstrates the genetic variation among sugarcane cross families. Additionally, these results demonstrate that the family cross with the highest rate of seedling survival does not necessarily yield the highest biomass. One example of this is cross 289 which had the lowest biomass of all the crosses despite having the highest survival.

Table 2.2. Analysis of variance of fixed effects for preemergence herbicide injury, seedling survival, and biomass for the sugarcane seedling family and PRE herbicide experiments conducted at the LSU AgCenter's Sugar Research Station in 2019 and 2020.

Source	Herbicide Injury (%)	Survival (%)	Biomass (Mg ha^{-1})
	-------	P-value	-------
Cross	0.7170	0.0095	<.0001
Herbicide	0.0004	0.0026	0.076
Cross X Herbicide	0.9553	0.4007	0.9189

Table 2.3. Sugarcane seedling survival rate and biomass means averaged across eight herbicide treatments for sugarcane seedling experiments conducted at the LSU AgCenter's Sugar Research Station in 2019 and 2020.

Cross	Survival[1] (%)	Biomass (Mg ha^{-1})
7	90 ab[2]	19.8 a[1]
138	92 a	17.7 abc
162	81 c	14.6 de
166	91 ab	16.9 bcd
170	87 abc	16.1 cde
192	86 bc	14.6 de
231	88 abc	16.8 cd
242	90 ab	18.1 abc
288	93 a	19.5 ab
289	93 a	13.6 e

[1]Sugarcane seedling survival evaluated 90 days after treatment.
[2]Means within a column followed by the same lowercase letter are not significantly different.

Herbicide treatment had a significant ($P \leq 0.05$) effect on crop (seedling) injury and seedling survival. The greatest level of injury was noted for the metribuzin treatment and averaged 3.4%, whereas the *S*-metolachlor at 1.05 kg ha^{-1} and the *s*-metolachlor + atrazine + mesotrione treatments averaged 1% or less injury at 7 DAT (Table 2.4). Sugarcane injury for the metribuzin treatment was noted as leaf tip necrosis. For injury, two treatments showed no significant difference when compared to the check, these were the low rate of *S*-metolachlor

(1.05 kg ha^{-1}) and *S*-metolachlor + mesotrione + atrazine (Lumax EZ) treatments. All other herbicide treatments yielded more injury than the untreated check. However, treatments that had a lower rate of injury did not have a competitive advantage for biomass. There was only one treatment which significantly decreased seedling survival 90 DAT as compared to the untreated check (Table 2.4). Only 83% of sugarcane seedlings survived the metribuzin treatment as compared to a 90% survival rate for the untreated check. However, anecdotal observations have shown the impact of metribuzin on sugarcane seedling survival when applied after the tillering stage to be negligible (M. Pontiff, Personal Communications December 15, 2021). Metribuzin at 1.68 kg ha^{-1} is commonly applied to sugarcane seedling fields 30 to 40 days after transplanting at the LSU AgCenter's Sugar Research Station (M. Pontiff, Personal Communications December 15, 2021). Once tillering occurs, seedlings have more vigor and a higher tolerance to applications of herbicides. This increased vigor allows for applications of metribuzin without significant seedling injury or mortality. First ratoon sugarcane seedlings also exhibit increased vigor, thus the weed management programs for these fields are the same as the commercial standard.

Table 2.4. Preemergence herbicide injury, sugarcane seedling survival, and biomass means averaged across ten sugarcane crosses for sugarcane seedling experiments conducted at the LSU AgCenter's Sugar Research Station in 2019 and 2020.

Treatment[1]	Rate (kg ha^{-1})	Injury[2] (%)	Survival[3] (%)	Biomass (Mg ha^{-1})
S-metolachlor	1.05	0.8 bc[4]	91 a	16.2
S-metolachlor	2.1	3.1 a	89 a	17.6
S-metolachlor + Atrazine + Mesotrione	1.05 + 0.394 + 0.249	1.0 bc	92 a	17.9
Mesotrione + Atrazine	0.105 + 1.68	1.9 ab	90 a	16.9
Metribuzin	1.68	3.4 a	83 b	16.8
Pendimethalin + Atrazine	0.93 + 2.24	2.1 ab	90 a	15.9
Pendimethalin + Atrazine	2.32 + 2.24	2.1 ab	88 a	16.4
Untreated Check		0 c	90 a	16.4

[1]Herbicide treatments were applied immediately following transplanting on April 30, 2019, and April 21, 2020.

[2]Crop injury evaluated 7 days after treatment (DAT); 0 to 100% scale, where 0% shows no distinguishable injury when compared to the check and 100% being complete sub-plot death.
[3]Sugarcane seedling survival evaluated 90 DAT.
[4]Means within a column followed by the same lowercase letter are not significantly different.

There are a limited number of herbicides available for use in sugarcane. Because of this some groups of herbicides are frequently used, this includes group 3 (pendimethalin, trifluralin), group 4 (2,4-D, dicamba), group 5 (atrazine, ametryn, metribuzin, diuron, hexazinone), and group 18 (asulam) (Odero et al. 2018). The addition of a new herbicide group in sugarcane (group 15, *S*-metolachlor) will allow sugarcane growers to use a more diverse rotation of MOAs. If sugarcane growers diversify their spray programs, there is a smaller chance of weeds developing herbicide resistance. While there are not many weed populations that have developed a resistance to *S*-metolachlor there are a few. Researchers in Arkansas have observed populations of palmer amaranth (*Amaranthus palmeri*) that have developed some resistance to *S*-metolachlor (Brabham et al. 2019). Additionally, some populations of waterhemp (*Amaranthus tuberculatus*) in Ohio have shown some level of resistance to *S*-metolachlor (Loux 2021).

S-metolachlor was only recently made available to the domestic sugarcane industry and published research on its effects on newly transplanted sugarcane seedlings is lacking. One of the main objectives of this study was to determine if this chemical would be a safe option for weed control in sugarcane seedling fields. Because all the treatments containing *S*-metolachlor did not have a negative effect on seedlings this chemical is a viable option for weed control in sugarcane seedling fields. *S*-metolachlor provides PRE control of yellow nutsedge and many annual grasses and broadleaf weeds (LSU AgCenter 2022).

Pendimethalin at 0.93 kg ha^{-1} plus atrazine at 2.24 kg ha^{-1} had been the standard herbicide treatment applied to newly transplanted sugarcane seedling fields at the LSU AgCenter's Sugar

Research Station until recently when the breeding program began using *S*-metolachlor at 1.05 kg ha^{-1}. This herbicide program shift was prompted from communication between the USDA-Houma breeding programs lead staff which reported improved browntop panicum (*Urochloa ramosa*) control in newly established sugarcane seedling fields with *S*-metolachlor (1.05 kg ha^{-1}) (K. Gravois, Personal Communications February 24, 2022). Whilst antidotal observations at the USDA showed no obvious injury to sugarcane seedling populations, no definitive data existed on the safety of *S*-metolachlor or other labeled herbicides on newly established sugarcane seedlings.

When developing a weed management strategy in newly established sugarcane seedling several considerations must be considered. While herbicide safety has been the predominant criterium utilized in herbicide selection for sugarcane breeding programs, other factors such as the targeted weed species and herbicide weed spectrum should be included prior to herbicide selection. Furthermore, herbicide rate greatly influences weed control. While pendimethalin at 0.93 kg ha^{-1} is safe to use on newly transplanted sugarcane seedlings, this rate is well below the recommended rate for commercial sugarcane production (2.24 to 3.36 kg ha^{-1}) (LSU AgCenter 2022). The results of this study revealed that 2.32 kg ha^{-1} of pendimethalin did not impact sugarcane seedling survival, thus this rate should be considered when developing weed management strategies for newly planted sugarcane seedling fields in the future.

The results from this experiment provide herbicide options which are safe to use on sugarcane seedlings following the transplanting procedure, and thus provides an impetus to develop herbicidal weed management strategies based upon the weed spectrum present in fields used for the establishment of sugarcane seedlings.

In conclusion all the PRE herbicides evaluated in this experiment excluding metribuzin were safe to use on sugarcane seedlings directly after they are transplanted into the field. This knowledge will allow researchers on the sugar research station to confidently apply these PRE herbicides without causing permanent damage or losses to sugarcane seedlings. Additionally, because multiple MOAs are safe to use on sugarcane seedlings a rotation of different active ingredients can be used. Rotating active ingredients or using a premix of different active ingredients can help prevent the development of herbicide resistant weeds.

Chapter 3. Preemergence Control of Seeding Johnsongrass

Introduction

Johnsongrass (*Sorghum halepense* L. Pers.) is a noxious weed with worldwide distribution that negatively affects the yield of many field crops; some examples include corn, soybean, sugarcane, and cotton. Johnsongrass can impact yield either by competing for resources, by acting as a host for numerous pathogen species, or by exuding allelopathic toxins from its roots (Parsons and Cuthbertson 1992; Vasilakoglou et al. 2005). In sugarcane johnsongrass has shown to cause up to an 84% reduction in yield and can cause some fields to be removed from production after only one year (Arevalo 1977; Millhollon 1970).

Johnsongrass is a course clumping grass which can grow up to 2 m in height. Johnsongrass leaf blades range from 20 to 60 cm long and 10 to 30 mm wide with fringed membranous ligules and a thick white midrib, both the leaf blade and sheath are mostly hairless except for sporadic hairs near the collar. Johnsongrass produces an open panicle seed head 12 to 15 cm long with numerous branches, the seed starts out green but changes to a dark red or purple upon maturity (Virginia Tech 2020). Johnsongrass can reproduce from either a seed or a subsurface rhizomatous root structure. One plant can produce up to 28,000 seeds in a year and can grow one meter of rhizomes in a day (Horrowitz 1973). Johnsongrass seed can remain dormant and viable in the soil for up to ten years which allows for continual reinfestation and a large build up in the weed seedbank (Huang and Hsiao 1987) (Johnson et al. 1997). Rhizomes can form 18 days after seedling emergence and can produce new plants even when cut away from the host plant (Horowitz 1972; McWhorter 1961).

Johnsongrass control in sugarcane is most effectively achieved through preventative measures. Preventative control of johnsongrass begins during fallow land preparation using a combination of mechanical and chemical control (Orgeron 2019). Tilling of the field can destroy existing johnsongrass populations. While tilling can break up the rhizomatous root structure of johnsongrass new plants can still form from fragments of rhizomes. To control these emerging plants application of glyphosate should be applied at 0.84 to 1.68 kg ha^{-1}. After planting has occurred, seedling johnsongrass can be controlled with the use of PRE herbicides.

There are many PRE herbicides which provide control of seedling johnsongrass some examples are clomazone, metribuzin, pendimethalin, trifluralin, and hexazinone + diuron. While these chemicals can control seedling johnsongrass they do not provide control of established or rhizomatous plants. Established johnsongrass can be controlled with an application of asulam at 3.75 kg ha^{-1}. This application must be done at a temperature of at least 15 C and when johnsongrass is between 30 to 45 cm in height.

Sugarcane varieties are developed at the LSU AgCenter's Sugar Research Station in St. Gabriel, LA. The first step in developing new varieties of sugarcane is to cross two existing varieties thus producing hybrid seed which is genetically unique. This seed is then grown in a greenhouse before being transplanted into a field. After a year these seedlings will be observed for continuation in the program. During this time the seedlings are subject to many environmental pressures including drought, flooding, freezes and weed pressure. Sugarcane seedlings grow slower than conventionally grown sugarcane therefore can be outcompeted more easily.

While several different weed populations are present in sugarcane seedling fields on the Sugar Research Station, johnsongrass is the most problematic. There are several preemergence (PRE) herbicides labeled for use in sugarcane which provide excellent control of seedling johnsongrass (LSU AgCenter 2022). However, anecdotal observations suggest that sugarcane seedlings are more susceptible to herbicidal injury than conventionally grown sugarcane. A separate study was conducted to determine which PRE herbicides are safe to use on sugarcane seedlings. The objective of this study was to determine the efficacy of commonly used PRE herbicides in controlling seedling johnsongrass.

Materials and Methods

Study Site and Experimental Design

Field experiments were conducted in 2020 at the LSU AgCenter's Sugar Research Station in St. Gabriel, Louisiana (30.267221 Lat –91.106431 Lon) to determine the efficacy of commonly used PRE herbicides in controlling seedling johnsongrass. Fifty-four (54) grams of johnsongrass seed were sown per plot on a raised bed in a commerce silty loam (*Fine-silty, mixed, nonacid, thermic Aeric Fluvaquent*) soil; pH of 7.3 and 1.3% organic matter. Plot size was 0.5 m^{-2} by 3 m^{-2} laid out in a randomized complete block design with four replications for each herbicide treatment. Planting occurred on May 25, 2020, and June 30, 2020. Following this a machine powered hand tiller was used to lightly incorporate the seed into the soil. Immediately following planting and tillage operations herbicide treatments were applied to their respective plot using a handheld CO_2 pressurized backpack sprayer equipped with AIXR 11002 flat fan nozzles (TeeJet® Technologies, Spraying Systems Co., Wheaton, IL 60187) calibrated to deliver 140 L ha^{-1}. Herbicide treatments were:

1: *s*-metolachlor (Medal II EC, 1.05 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409),

2: *s*-metolachlor (Medal II EC, 2.10 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409), 3: *s*-metolachlor + atrazine + mesotrione (Lumax EZ, 1.55 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409) at,

4: mesotrione (Callisto 0.105 kg ha^{-1}; Syngenta Crop Protection, P.O. Box 18300 Greensboro, NC 27409) + atrazine (Atrazine 4L, 1.68 kg ha^{-1}; Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632),

5: metribuzin (TriCor 75DF 1.68 kg ha^{-1}; UPL NA Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406),

6: pendimethalin (Satellite Flex, 0.93 kg ha^{-1}; UPL NA Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406) + atrazine (Atrazine 4L, 2.24 kg ha^{-1} ; Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632),

7: pendimethalin (Satellite Flex, 2.32 kg ha^{-1}; UPL NA Inc., 630 Freedom Business Center, Suite 402, King of Prussia, PA 19406) + atrazine (Atrazine 4L, 2.24 kg ha^{-1} ; Loveland Products, Inc. P.O. Box 1286, Greeley, CO 80632), and

8: untreated check.

Data Collection

A 30 cm ring was randomly placed in each plot to demarcate a representative sample. From this area, seedling johnsongrass density and dry weight were recorded. At 7, 14, and 28 days after treatment (DAT) all emerged johnsongrass seedlings within each ring were counted and recorded. After 28 days, all johnsongrass plants within the rings were harvested and weighed. These samples were then oven dried at $59^{O}C$ for 48 hr to calculate johnsongrass dry weight.

Statistical Analysis

Johnsongrass seedling counts, and dry biomass were analyzed using the MIXED procedure in SAS (release 9.4, SAS Institute, Cary, NC) to determine statistical differences among treatments. PROC UNIVARIATE was used to test for normality and means were separated using the Tukey range test ($P<0.05$). Herbicide treatment was considered a fixed effect and time (run) and replication were considered random effects.

Results and Discussion

Johnsongrass emergence was observed at 7, 14, and 28 DAT and biomass was gathered at 28 DAT, results are shown in Table 3.1. At 7 and 14 DAT, the pendimethalin + atrazine (2.32 + 2.24 kg ha^{-1}) treatment provided the best control of johnsongrass, with an average of 4 and 7 johnsongrass plants per plot, respectively. However, at 28 DAT the number of johnsongrass plants in the metribuzin treatment was greatly reduced from the 7 and 14 day observations and averaged 2 plants per plot. While the metribuzin treatment provided the greatest numerical level of control of seedling johnsongrass at 28 DAT the *S*-metolachlor (2.1 kg ha^{-1}), *S*-metolachlor + mesotrione + atrazine, and both pendimethalin + atrazine treatments showed statistically similar results to metribuzin. The metribuzin and pendimethalin + atrazine (2.32 + 2.24 kg ha^{-1}), treatments had significantly fewer johnsongrass seedlings at 28 days and averaged 2 and 4 plants, respectively, as compared to the untreated check which averaged 35 seedling johnsongrass plants. This delayed control in metribuzin treatment is likely due to the mode of action (MOA) of metribuzin which is a photosystem II inhibitor. The metribuzin and pendimethalin + atrazine (2.32 + 2.24 kg ha^{-1}) treatments also had the lowest johnsongrass dry weight and averaged 0.051 and 0.499 g, respectively.

The only treatment which did not provide any control of johnsongrass populations was the mesotrione + atrazine treatment. This is not an unexpected result as both mesotrione and atrazine predominantly provide control of broadleaf weeds. This treatment was included in this experiment because it contains two of the three active ingredients in Lumax EZ (*S*-metolachlor + mesotrione + atrazine). Prior to this experiment no published research was available as to the effect of *S*-metolachlor on sugarcane seedlings. If Lumax EZ did have an antagonistic effect on sugarcane seedlings this treatment would have helped determine if this effect was due to the inclusion of *S*-metolachlor or not. Fortunately, Lumax EZ did not have an antagonistic effect on sugarcane seedlings and remains a viable weed control option for sugarcane seedling fields.

Table 3.1. Mean number of johnsongrass plants at 7, 14, and 28 days after treatment (DAT) and dry weight of johnsongrass for experiments conducted at the LSU AgCenter's Sugar Research Station on May 25, 2020, and June 30, 2020.

Treatment	Rate (kg ha^{-1})	7 DAT		14 DAT		28 DAT		Dry wt. (g)	
S-metolachlor	1.05	20	ab	20	bc	16	b	3.860	b
S-metolachlor	2.1	13	bc	12	cd	5	bc	1.218	bc
S-metolachlor + Atrazine + Metribuzin	1.05 + 0.394 + 0.249	15	abc	13	cd	10	bc	2.479	bc
Mesotrione + Atrazine	0.105 +1.68	24	ab	32	ab	33	a	8.791	a
Metribuzin	1.68	26	a	21	bc	2	c	0.051	c
Pendimethalin + Atrazine	0.93 + 2.24	15	abc	15	cd	11	bc	1.584	bc
Pendimethalin + Atrazine	2.32 + 2.24	4	c	7	d	4	c	0.499	c
Untreated Check		27	a	34	a	35	a	11.671	a
P-value		0.0103		0.0002		<.0001		<.0001	

[1] Means within a column followed by the same lowercase letter are not significantly different.

Johnsongrass can produce up to 28,000 seeds per plant in one year which can allow for a large buildup of johnsongrass seed in the weed seedbank (Horrowitz 1973). Additionally, johnsongrass seed can remain dormant and viable in the soil for up to 10 years which allows for continuous emergence year after year (Johnson et al. 1997). The use of PREs can help control seedling johnsongrass establishment and prevent further infestations. Some of the PRE treatments used in this experiment provided adequate control of seedling johnsongrass.

The treatments which provided the best control of seedling johnsongrass in this experiment were the metribuzin and pendimethalin + atrazine (2.32 + 2.24 kg ha^{-1}) treatments. These results provide valuable insight into which PREs used in commercial sugarcane production provides effective control of seedling johnsongrass.

Chapter 4. Summary

Sugarcane varieties are developed on the LSU AgCenter's Sugar Research station in Saint Gabriel, Louisiana. New sugarcane varieties are produced by crossing existing varieties to produce true seed. This seed is grown in a greenhouse until it can be transplanted into a field. Sugarcane seedling growth is slower and more inconsistent than conventional sugarcane production and is therefore more susceptible to weed interference. Additionally, anecdotal observations suggest that sugarcane seedlings are more susceptible to herbicidal injury than conventional sugarcane. The objectives of this research were: 1. to determine if the PRE herbicides commonly used in conventional sugarcane production are safe to use on sugarcane seedlings when applied directly after being transplanted into the field and 2. to determine the efficacy of commonly used PRE herbicides in controlling seedling johnsongrass. The herbicide treatments evaluated were:

1. (*S*-metolachlor 1.05 kg ha^{-1})
2. (*S*-metolachlor 2.1 kg kg ha^{-1})
3. (*S*-metolachlor 1.05 kg ha^{-1}+ Atrazine 0.394 kg ha^{-1} + Mesotrione 0.249 kg ha^{-1})
4. (Mesotrione 0.105 kg ha^{-1}+ Atrazine 1.68 kg ha^{-1})
5. (Metribuzin 1.68 kg ha^{-1})
6. (Pendimethalin 0.93 kg ha^{-1} + Atrazine 2.24 kg ha^{-1})
7. (Pendimethalin 2.32 kg ha^{-1} + Atrazine 2.24 kg ha^{-1})
8. Untreated check

S-metolachlor was recently labeled for use in sugarcane and thus little to no research has been conducted on its effects on sugarcane seedlings. Other common PRE herbicides used in this experiment were metribuzin and pendimethalin. A low rate of pendimethalin (0.93 kg ha^{-1}) has previously been used in sugarcane seedling fields with no negative effects on seedling survival. This rate of pendimethalin is well below the recommended rate for commercial sugarcane production (2.24 to 3.36 kg ha^{-1}) which is needed to gain adequate control of problematic weeds such as seedling johnsongrass and itchgrass. This study showed pendimethalin at 2.32 kg ha^{-1} had no impact on sugarcane seedling survival and should be considered as a viable herbicide treatment. Metribuzin has previously been used in sugarcane seedling fields; however, it was not applied directly after transplanting but instead applications were made after tillering had occurred (30-40 days after planting). The results from the first experiment showed that all treatments excluding metribuzin did not have a significant impact on sugarcane seedling survival. Sugarcane seedling survival was the lowest for the metribuzin treatment and averaged 83%. Results from this experiment showed *S*-metolachlor and pendimethalin are both safe to use at higher rates (*S*-metolachlor at 2.10 kg ha^{-1}, pendimethalin 2.32 kg ha^{-1}) on sugarcane seedlings following the transplanting procedure as compared to the low standard rates (*S*-metolachlor at 1.05 kg ha^{-1}, pendimethalin 0.93 kg ha^{-1}) of these products which have been utilized in the establishment of sugarcane seedlings at the LSU AgCenter's Sugar Research Station. This will allow staff on the Sugar Research Station to confidently apply these products without fear of reducing seedling survival. Additionally, a rotation of these herbicides can be used, which can help provide broader weed control and prevent herbicide resistant weeds from developing. Herbicidal rotation can also provide a broader level of weed control, for example, *S*-metolachlor provides control over yellow nutsedge while pendimethalin provides control of itchgrass (LSU AgCenter 2022).

The second experiment tested the efficacy of these herbicides in controlling seedling johnsongrass populations. Results revealed that the metribuzin and pendimethalin + atrazine

(2.32 + 2.24 kg ha^{-1}) treatments provided the best control of seedling johnsongrass emergence and had the lowest seedling johnsongrass dry weight at 28 days after treatment (DAT). Both of these treatments reduced johnsongrass dry weight by over 95% when compared to the untreated check. Other treatments which provided some suppression of johnsongrass were *S*-metolachlor (2.1 kg ha^{-1}), *S*-metolachlor + Atrazine + Mesotrione (Lumax EZ), and Pendimethalin + Atrazine (0.93 +2.24 kg ha^{-1}) and reduced johnsongrass dry weight by 90%, 79%, and 86%, respectively as compared to the check.

Based on the results from these experiments a new herbicide program was suggested for use on sugarcane seedling fields on the Sugar Research Station in St. Gabriel, LA. The recommendation is to apply pendimethalin at 2.32 kg ha^{-1} directly after the seedlings are transplanted into the field. A follow up application of metribuzin at 1.68 kg ha^{-1} should be applied after tillering has occurred. If further weed control is necessary *S*-metolachlor can be applied at 2.1 kg ha^{-1}. While pendimethalin was used in previous herbicide programs for sugarcane seedling fields on the Sugar Research Station, it was used at a lower rate and did not provide adequate control of johnsongrass.

The herbicide program developed from this experiment is safe for use in sugarcane seedling fields while providing the best control of competing johnsongrass populations. This herbicide program will allow for seedlings to grow to their full potential, thus allowing researchers to make accurate observations on seedling growth. A well-managed weed population will prevent the unnecessary elimination of sugarcane seedlings that could have the genetic potential to become the next leading variety.

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Vita

Carleton Blake Baucum is the son of Heather and Leslie Baucum. Carleton was raised in Clewiston, Florida where he graduated from Clewiston High School in 2010. In the fall of 2010, he enrolled in South Florida Community College where he received his Associate in Arts degree in General Agriculture in 2012. In the fall of 2012, he enrolled at the University of Florida and began working on his Bachelor of Science degree in Plant Science Sustainable Food Production. He graduated in 2015 and began working as a production supervisor for the United States Sugar Corporation. In 2017 Carleton accepted a position as a research associate in the Louisiana State University AgCenter's sugarcane variety development program. In 2018 he enrolled at Louisiana State University in the department of Plant, Environment, and Soil Sciences under the direction of Dr. Albert Orgeron and Dr. Lauren Lazaro. Carleton plans to graduate in May of 2022 with his Master of Science in Weed Science.