EVALUATION OF HERBICIDES ON THE ESTABLISHMENT OF PEARL MILLET [PENNISETUM GLAUCUM (L.) R.BR.] X NAPIERGRASS (PENNISETUM PURPUREUM SCHUMACH.)

by

GREGORY BRYAN WILSON

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of committee,	Russell W. Jessup
Committee members,	Byron L. Burson
	Paul A. Baumann
Head of Department,	David D. Baltensperger

August 2017

Major Subject: Agronomy

ABSTRACT

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] x napiergrass (*Pennisetum purpureum* Schumach.) (PMN) hybrids have potential as a seeded, perennial bioenergy or forage crop. The PMN hybrid utilized in this study (PMN10TX13) was developed as an alternative to herbaceous bioenergy feed stocks that either require vegetative propagation, complicated planting strategies due to small seed size, or annual reseeding due to annual growth habit. However, PMN seedlings are impacted by competition from nearby weeds for water, nutrients, and sunlight during establishment. To date, there is limited agronomic information on strategies for effective establishment of weed-free PMN stands. The objective of this study was to develop herbicide response tests to determine the phytotoxic effects of selected pre-crop emergence (PRE) and post-crop emergence herbicides (POST) on the seeded establishment of PMN. Several herbicides with utility for weed free establishment of seeded PMN were successfully identified. Pre-emergent herbicides Balance Pro (isoxaflutole), Dual II (s-metolachlor,) Plateau (imazapic), and Permit (halosulfuron) were effective as long as sufficient rates of seed safener were used. Post-crop emergent herbicides Permit (halosulfuron), Prowl (pendimethalin), Banvel (dicamba), Aatrex (atrazine), AIM (carfentrazone-ethyl), Warrant (achetochlor), and Huskie (pyrasulfotole) were also found to be safe for use at the 5-7 leaf stage and beyond.

ACKNOWLEDGEMENTS

I would like to thank God and my family, without either I would be lost. I would like to thank my committee; I admire their dedication, work ethic, and leadership during the course of my study. I would also like to thank Dr. Harry Cralle for his passionate inspiration and for all of our conversations.

CONTRIBUTORS AND FUNDING SOURCES

This work was supervised by a thesis committee consisting of Dr. Russell Jessup [Advisor] and Dr. Paul Baumann [Committee Member] of the Department of Soil and Crop Science and Dr. Byron Burson [Outside Department Committee Member] of the Department of Molecular & Environmental Plant Sciences and USDA-ARS.

All work for the thesis was completed independently by the student and graduate study was supported by a Graduate Assistantship from Texas A&M University Department of Soil and Crop Science.

NOMENCLATURE

DAT	Days after treatment
PMN	Pearl Millet x Napiergrass
Phytotoxicity	Plant injuries occurring after chemicals are utilized to protect plants from pests, to fertilize plants, or to regulate plant growth.
OTT	Herbicide is applied over-the-top of the canopy directly on the foliage using liquid carbon dioxide pressurized sprayer.
PRE	Herbicide that is formulated to control weed sp. before it emerges.
POST	Herbicide that is formulated to control weed sp. after it emerges.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

CHAPTER I

INTRODUCTION

Projections by the Intergovernmental Panel on Climate Change include higher levels of global temperatures and atmospheric CO_2, which have the potential of negatively impacting yields of major food crops (IPCC 2014). This poses a larger food security risk to C_3 grain crops such as rice (*Oryza sativa* L.) and wheat (T*riticum aestivum* L.) a because their C_3 photosynthetic pathway would assimilate CO_2 at lower rates under heat stress and high levels of atmospheric CO_2. Comparatively, drought tolerant gramineous crops which utilize the C_4 pathway will become increasingly important for sustained agricultural productivity worldwide. One C_4 crop with potential as a highly productive forage and biofuel feedstock is the interspecific hybrid between pearl millet [*Pennisetum glaucum* (L.) R. Br.] and napiergrass (*P. purpureum* Schumach.) (PMN). Presently, only limited agronomic information exists regarding the safe, or weed-free, establishment of PMN. This study was conducted to determine the phytotoxic effects of selected pre-crop emergence (PRE) and post-crop emergence (POST) herbicides on the establishment of PMN from seed. These findings will be used to develop a protocol whereby stands of PMN hybrids can be established by planting seed with minimal competition from competing weeds.

OBJECTIVES

The objective of this investigation was to determine the phytotoxic effects of various PRE and POST herbicides on seeded PMN establishment by:

1. Observing and measuring the effects of selected pre- and post-crop-emergent herbicides applied over-the-top to establish documented activity.
2. Observing the effects of selected pre-crop-emergent herbicides on seed germination when applied to the soil pre-plant, in order to establish documented activity for the use of pre-crop emergent herbicides in a seeded PMN field cropping system.
3. Utilizing a broad spectrum regime of chemistry for PMN establishment and maintenance that effectively targets a variety of weeds and seasonal timing for PMN establishment and maintenance.
4. Observing the effect of varied rates of glyphosate (RoundUp Max) when used to eradicate senescing PMN hybrids in the field.

CHAPTER II

LITERATURE REVIEW

The Poaceae is a large family of monocotyledonous flowering plants called grasses that are nearly ubiquitous across terrestrial global ecosystems (Gould 1983). Within the grasses, there are two highly distinct groups: the first, commonly called the BOP clade, represents grasses utilizing C_3 photosynthesis, (subfamilies Oryzoideae, Bambusoideae, and Pooideae) and the second, commonly called the PACMAD clade, representing grasses utilizing C_4 photosynthesis, (subfamilies Aristidoideae, Panicoideae, Arundinoideae, Micraioideae, Danthonioideae, and Chloridoideae). In a recent study (Soreng et al. 2015) of both frequency and timing of the evolution of the C_4 photosynthetic pathway, a maximum likelihood tree was created to represent the phylotaxonomy of grasses; this tree identified 12 subfamilies, 51 tribes, and 80 subtribes, with 41% of the 12,074 gramineous spp. utilizing the C_4 photosynthetic pathway worldwide. The genus *Pennisetum* is within the C_4 group and includes pearl millet and its close relative napiergrass. These two grasses are geographically similar, not reproductively isolated, and subunits of the same gene pool (Brunken 1977). This genus is a heterogeneous assemblage of species with different basic chromosome numbers (x = 5, 7, 8, and 9), ploidy levels (diploid to octoploid), reproductive behaviors (sexual or apomictic), and life cycles (annual, biennial, or perennial) (Stapf and Hubbard 1934: Gould 1983).

Pearl millet is a tropical annual bunchgrass, belonging to the PACMAD clade of grasses that utilize the C_4 photosynthetic pathway. This photosynthetic type generally possesses higher proportions of vascular tissues, photosynthetic efficiency, and drought and heat resistance than grasses with the C_3 pathway (Nelson and Moser 1994). Pearl millet is generally grown as a grain, forage, or multi-use crop, and it is related to other important grasses globally including corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.), and sugarcane (*Saccharum officinarum* L.) (Gulia et al. 2006).

Napiergrass is a warm-season perennial species that belongs to the PACMAD clade, and it has reported chromosome numbers of 2n=27, 28, and 56 (Fedorov 1974). This species is typically grown as forage because of its drought tolerance, adaptation to a wide range of soil types, high photosynthetic rate, and water-use-efficiency (Anderson et al. 2008). Napiergrass plays an important role in the tropics to the small land holder dairy farmers because of its high nutritive value for dairy cattle, particularly when supplemented with high quality feeds such as legumes (Nyambati et al. 2003). The grass constitutes up to 80% of forage for smallholder dairy farms in South Africa (Staal et al. 1987). It is also important as beef cattle feed because of the ease of managing the grass in cut-and-carry systems. It is considered an invasive species in some tropical regions because of its rapid growth rate and potential for both vegetative and seed propagules. In the United States, authorities in the state of Florida have added the grass to the invasive species list for that state (Florida Exotic Pest Plant Council 2005). The chemical composition of napiergrass varies and typically contains 20-60% digestible material, 7 - 20% crude protein, 70% neutral detergent fiber, and 45% acid detergent fiber (Hanna 1980: Gwayumba et al. 2002: Aganga 2005).

Pearl millet and napiergrass can be successfully hybridized to produce vigorous interspecific hybrids (Burton 1944) that have high yield potential (Hanna et al. 2004) because of their capacity to produce more tillers, leaves, and biomass than either parent (Gupta and Mhere 1997). It is also important to note that the F_1 PMN hybrids are sterile triploids (2n=3x=21) that produce no fertile seed and are considered non-invasive (Jessup 2013). Being derived from fertile parents, F_1 PMN hybrids are propagated by seed which can significantly reduce labor and establishment costs versus the typically clonally propagated napiergrass. In addition to high biomass accumulation, PMN is drought tolerant, resistant to numerous biotic stresses, and possesses protein concentrations similar to that of its parent species (Bora 2012: Turano 2016).

POTENTIAL AS A FORAGE SOURCE

According to the National Institute for Animal Agriculture's (2013), the demand for animal protein in the next 38 years will increase significantly. Economists estimate that by 2050, global meat production must increase by 73% to meet the expected 43% increase in world population. PMN hybrids could play a major role in producing high quality forage for the cattle industry because their dry matter content can be higher than that of traditional napiergrass varieties; however, dry matter content, lignin content, and nutrient content of PMN varieties can vary significantly depending on age, seasonality, landscape, plant variety, and crop management strategy (Ogoshi et al. 2010: Rengsirikul et al. 2011: Xie et al. 2011: Turano 2016). In a recent study conducted in Hawaii, yield and nutritive value of PMN varied significantly depending on irrigation frequency and cultivar used (Turano 2014); despite this, PMN hybrids outperformed napiergrass varieties and other grasses for yield, nutritional content, and digestibility. An additional benefit of a PMN forage system is that its late growth provides high quality forage during a period of the year when most warm-season grasses have declined in quality and yield (Hanna 1980).

POTENTIAL AS A CELLULOSIC BIOFUEL FEEDSTOCK

Napiergrass has been reported to produce as much as 100 tons of fresh biomass hectare^{-1} and has one of the fastest growth rates of all higher plants (ASARECA 2011). This makes it a potential candidate for the production of cellulosic ethanol (Houghton et al. 2006). Extracting cellulosic ethanol involves various enzymes which increase porosity of biomass particles and increase the accessibility to cellulose (Carroll and Somerville 2008), the converted glucose can be separated from the remaining solid waste and ethanol is the derived product from the same fermentation process as corn-based bioethanol systems (Verma et al. 2013). Currently, the United States is the world's largest ethanol producer (Hettinga 2008: Correll 2014) and models show the production of alcohol with sugarcane to have a positive net energy balance, except when a production system is entirely based on fossil fuel energy (Hopkinson 1980). According to the Energy Policy Act of 2005, revised under the Energy Independence and Security Act of 2007, the EPA's Renewable Fuel Standard requires 60 gigaliters of renewable fuel to be blended into transportation fuel by 2022. Unfortunately, over the past 4 years the cellulosic advanced biofuels have only produced 1.4 gigaliters over those 4 years which is small fraction of the original cellulosic mandate (Environmental Protection Association 2015). In order to meet the original mandate for cellulosic fuels there will need to be improvements in feedstock logistics as well as our agronomic knowledge of cellulosic cropping systems (EISA 2007). PMN serves as a non-invasive alternative to napiergrass that can be utilized in multiple biomass-based production systems. The high biomass production potential and reduced environmental risks could avoid significant carbon debt, provide necessary material for renewable fuels, and provide substantial greenhouse gas emission mitigation (Jessup 2013).

HERBICIDE USE IN WEED MANAGEMENT

PMN is a non-invasive alternative to napiergrass that can be utilized in multiple biomass-based production systems. Since cropping systems possess varying environmental conditions and weed pressures, and since there are no herbicide reference labels outlining the effect to the PMN crop specifically, we will characterize different herbicide chemistries to document the phytotoxic effect on establishing PMN stands.

Stands of pearl millet and PMN are established by planting seed; whereas, stands of napiergrass are established by planting vegetative culms or billets. Similar to other seeded hybrid crops, seeded PMN requires time to close the canopy and outcompete nearby weeds (Jessup unpublished data); early season control of weeds is therefore important for successful plant establishment. Despite this need, efficacy of herbicides has not been reported directly in PMN. Carson (1987) reported that pearl millet grain yield was reduced by 36% from competition with weeds during two to seven weeks after pearl millet seedling emergence. Large scale production of pearl millet is possible only if selective herbicides are applied at planting and up to six weeks after planting to prevent yield losses (Ndahi 1981). Chemical control of broadleaved species in pearl millet has been obtained with atrazine (Ndahi et al. 1980: Jain et al. 1971), 2, 4-D (Farinelli et al. 2005: Pacheco et al. 2007: Dans 2010) and carfentrazone-ethyl (Lyon et al. 2007: Stahlman 2009). For best results, weed control should be performed early for seeded millets since the plant can only tolerate the presence of weeds until they are 15-20 cm tall (Berglund 1998).

Once napiergrass is established, annual weed control is normally not an issue because it out-competes other plants (Wright 1994) and once stands become established weeds are typically suppressed by leaf litter and coppice regrowth which will close the canopy faster than on the initial cycle (Turhollow 1994).

There are reports identifying herbicides that will control napiergrass to reduce its potential invasive spread. Cutts (2007) studied the effect of atrazine clomazone, diuron, pendimethalin, metribuzin, sulfentrazone, flumioxazin, ametryn, s-metolachlor, mesotrione, tembotrione, hexazinone, and terbacil to suppress unwanted growth. In order to limit future invasion of napiergrass escapes in sugarcane and vegetables, the response of newly established napiergrass plants to glyphosate, clethodim, sethoxydim, asulam, and trifloxysulfuron was determined using dose–response curves (Odero and Gilbert 2012). Control of napiergrass was found to be difficult in both of the before mentioned studies.

Currently, there is no evidence of the susceptible time period or the overall herbicide safety on seeded PMN; therefore, this study was designed to observe the effects of pre-crop emergence herbicides and post-crop emergence herbicides on the development of PMN seedlings and overall stand establishment.

CHAPTER III

MATERIALS AND METHODS

Three greenhouse experiments and two field experiments were conducted to observe phytotoxic responses of seeded PMN to selected PRE and POST herbicides applied to foliage and one greenhouse experiment to observe response to selected PRE herbicide and safener combinations applied to soil.

HERBICIDE CATEGORIZATION AND SELECTED HERBICIDES

[WSSA Group Number, Chemical Name (Trade Name)]

Many herbicides which are currently registered for the establishment of sugarcane, pearl millet, and other *Poaceae* crops have potential for use in napiergrass because of morpho-physiological similarities. The following subset was evaluated in this study according to the Herbicide Handbook (Shaner 2014).

Group 2

Imazapic and halosulfuron (Plateau and Permit): imidazolinones and sulfonylureas respectively, inhibit acetolactate synthase (ALS) production, also called acetohydroxyacid synthase (AHAS), a key enzyme in the biosynthesis of the branched-chain amino acids isoleucine, leucine, and valine. The herbicide blocks the in-plant production of these amino acids, and the plant subsequently dies from insufficient branched chain amino acids. Plateau is applied post-crop emergence and is used to provide pre and post-weed emergent control of many annual and perennial grasses including: *Panicum spp.*, johnsongrass (*Sorghum halepense* L)., goosegrass (*Eleusine indica* L.), broadleaf signal grass [*Brachiaria platyphylla* (Griseb.) Nash.], foxtail spp. (Setaria), and crabgrass spp. (Digitaria), and purple nutsedge (*Cyperus rotundus* L.) and yellow nutsedge (*Cyperus esculentus* L.). It also controls many annual broadleaf weeds such as sicklepod (*Senna obtusifolia* L.), morningglory spp. (Ipomoea), cocklebur spp. (Xanthium), and bristly starbur (*Acanthospermum hispidum* DC.). Permit is applied early pre-plant surface, pre-plant incorporated, and post-crop emergence in many production systems, such as: corn (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), soybean (*Glycine max* L.), wheat, rice, okra (*Abelmoschus esculentus* L.), some fruiting vegetables, and some fruit trees. Permit controls many annual broadleaf weeds including velvetleaf (*Abutilon theophrasti* Medik.), various amaranth spp. (*Amaranthus*), cocklebur, horsetail (*Equisetum*), jimsonweed (*Datura stramonium* L.), joint-vetch (*Aeschynomene virginica* L.), lambsquarter (*Chenopodium album* L.), common mallow (*Malva neglecta* Wallr.), tall morningglory [*Ipomoea purpurea* (L.) Roth], wild mustard (*Sinapis arvensis* L. ssp. arvensis), redroot pigweed (*Amaranthus retroflexus* L.), purslane (Portulaca L.), giant ragweed (*Ambrosia trifida* L.), hemp sesbania (*Sesbania herbacea* Mill. McVaugh), and shepherds purse [*Capsella bursa-pastoris* (L.) Medik.].

Group 3

Pendimethalin (Prowl): dinitroaniline herbicides bind to tubulin, the major microtubule protein. The herbicide-tubulin complex inhibits polymerization of microtubules at the assembly end of the protein-based microtubule but has no effect on depolymerization of the tubule on the other end, leading to a loss of microtubule structure and function. This herbicide is applied as pre-plant, pre-plant incorporated, early post-crop emergence and late post-crop emergence in various production systems. Prowl is labeled for crops such as: alfalfa (*Medicago sativa* L.), field and sweet corn, grain sorghum, rice, soybeans, cotton (*Gossypium hirsutum* L.), wheat, potatoes (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), sugarcane, sunflowers (*Helianthus annuus* L.), and several beans. It controls annual grass weeds including barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], crabgrass, shattercane (*Sorghum* spp.), foxtail spp., and goosegrass. It also controls some broadleaf weeds such as: lambsquarter, redroot pigweed, and velvetleaf.

Group 4

Dicamba (Banvel): benzoic acids are herbicides that act similar to endogenous auxin although the true mechanism is not well understood. The specific cellular or molecular binding site relevant to the action of endogenous auxin and the auxin-mimicking herbicides has not been identified. Synthetic auxins mimic the natural plant hormone Indole-3-acetic acid. These herbicides affect cell wall plasticity and nucleic acid metabolism, which leads to inhibited cell division and growth in meristematic regions. The herbicide is primarily applied post-weed emergence but provides residual control of germinating weeds. It is also used as a pre-plant and post-crop emergence in various production systems and can be used with a seed safener (Isoxadifen) to avoid seedling injury. Dicamba is labeled for crops such as: corn, sorghum, small grains, pasture, turf and rangeland-grasses. It primarily controls annual broadleaf weeds such as pigweed spp., lambsquarter, Canada thistle [*Cirsium arvense* (L.) Scop.], wild mustard, nightshade (*Solanum* L.), vetch, and field bindweed (*Convolvulus arvensis* L.).

Group 5

Atrazine (Aatrex): triazines are selective herbicides that inhibit photosynthesis by binding to the secondary quinone electron acceptor of photosystem II (Q_b) binding niche on the D1 receptor protein of the photosystem II complex in chloroplast thylakoid membranes. Herbicide binding at this protein location blocks electron transport from primary D2-bound quinone electron acceptor (Q_a) to Q_b and stops CO_2 fixation and production of ATP and NADPH2 which are all needed for plant growth; however, plant death occurs by other processes in most cases. Aatrex is primarily applied to both soil and foliage, early pre-plant, pre-plant incorporated, pre- and post-crop emergence. Atrazine is labeled for crops such as: corn, sorghum, municipal roadsides, sugarcane, small grains, pasture, turf and rangeland. It controls many annual broadleaf weeds such as pigweed spp., morning-glory, mustard, smartweed (*Polygonum pensylvanicum* L.), Canada thistle, cocklebur, and lambsquarter. It also controls some grass weeds such as wild oats (*Avena fatua* L.), crabgrass, barnyardgrass and foxtail spp.

Group 7

Substituted Urea diuron, (Direx): has the same mode of action as Group 5. Direx is applied pre- and post-crop emergence and is labeled for crops such as: alfalfa, sugarcane, winter barely (*Hordeum vulgare* L.), sprigged Bermudagrass [*Cynodon dactylon* (L.) Pers.], and perennial grass seed crops. It selectively controls many annual and perennial grasses and broadleaf weeds such as: crabgrass, barnyard grass, pigweed spp., purslane, velvetgrass (*Holcus lanatus* L.), foxtail spp., wild mustard, lovegrass (*Eragrostis* von Wolf), ryegrass (*Lolium perenne* L.), orchardgrass (*Dactylis glomerata* L.), sand-bur (*Cenchrus* L.), and sheperdspurse. A very high rate of this herbicide was chosen for two reasons, to verify application methods with a rate which would assuredly injure a grass plant and to compare the higher rate for use during eradication of PMN fields.

Group 14

Carfentrazone-ethyl (AIM): these herbicides inhibit the photoporphyrinogen oxidase (PPO), an enzyme that is responsible for chlorophyll and HEME biosynthesis. PPO inhibition leads to accumulation of PPIX (protoporphyrin IX) which creates free radical oxygen in the cell and destroys cell membranes. AIM is a contact herbicide that is applied post-crop emergence to monocot crops, typically as a fallow / burndown herbicide, including sugarcane, winter wheat and barley, turf, range, seed corn, silage corn, grain sorghum, and rice It selectively controls a wide spectrum of broadleaf weeds; treated plants will become necrotic and die shortly after contact.

Group 15

S-metolachlor and acetochlor, (Dual II Magnum, Warrant): These acetamide formulations are examples of herbicides that are currently thought to inhibit very long chain fatty acid synthesis. Susceptible plants fail to emerge or plant tissues are severely malformed. Dual is a selective herbicide recommended as a pre-plant surface-applied, pre-plant incorporated or pre-emergence herbicide, and a safener (fluxofenim) is generally used to avoid injury to target crop. It is used in corn (all types), cotton, peanuts (*Arachis hypogaea* L.), pod crops, potatoes, safflowers (*Carthamus tinctorius* L.), grain or forage sorghum, and soybeans. It selectively controls most annual grasses and broadleaf weeds including barnyardgrass, foxtail spp., red rice88f (*Oryza sativa* L.), yellow nutsedge, crabgrass (purple, white) signalgrass, Eastern black nightshade, fall panicum (*Panicum dichotomiflorum* Michx.), cupgrass [*Eriochloa villosa* (Thunb.) Kunth], wild proso millet (*Panicum miliaceum* L.), pigweed, goosegrass, witchgrass (*Panicum capillare* L.), and tall waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer.]. Warrant is a selective herbicide recommended as a pre-plant incorporated or pre-emergence herbicide. It is labeled for use in corn and controls most annual grasses and some annual broadleaf weeds. Annual grass weeds controlled include barnyardgrass, crabgrass, cupgrass, goosegrass, johnsongrass seedlings, *Panicum* spp., and wild oat. Annual broadleaf weeds controlled include purslane, prickly Sida (*Sida spinosa* L.), carpetweed (*Mollugo verticillata* L.), and dixie ticktrefoil [*Desmodium tortuosum* (Sw.) DC].

Group 27

Pyrasulfotole and isoxaflutole (Huskie and Balance Pro), isoxazoles and pyrazoles are examples of herbicides that inhibit p-hydroxyphenyl pyruvate dioxygenase, which converts p-hydroxymethyl pyruvate to homogentisate. This is a key step in plastoquinone biosynthesis and its inhibition gives rise to bleaching symptoms on new growth. These symptoms result from an indirect inhibition of carotenoid synthesis due to the involvement of plastoquinone as a cofactor of phytoene desaturase. Huskie is a selective post-weed emergence herbicide used for the control of certain broadleaf weeds in wheat, barley, conservation reserve programs, rye, grain sorghum, and triticale. Huskie controls over 50 broadleaf weeds including nightshade spp., mustard spp., field bindweed, pale smartweed, common mallow, and henbit. Balance Pro is a selective pre-crop emergence herbicide used for the pre-emergence control of certain broadleaf weeds in sugarcane, commonly used with the safener (cyprosulfamide) to avoid injury to crop (Robinson, 2013). Similar to Huskie, it controls over 50 broadleaf weeds.

APPLICATION TIMING

1. Pre-plant, pre weed-emergent or pre crop-emergent to surface - Herbicide applied to prepared bare soil before crop has been seeded or before crop emergence (timing based on label).
2. Pre-plant soil incorporated - Herbicide applied over-the-top of prepared bare soil, subsequently incorporated manually or mechanically into top-soil.
3. Post-crop emergent - Herbicide is applied over-the-top of crop foliage, based on growth stage of crop and target weed.

Table 1. Selected pre/post-crop-emergence herbicides used for study

Chemical family	Active ingredient	Trade name	Labeled rate $acre^{-1}$	A. i. $acre^{-1}$	A. i. $hectare^{-1}$
				lb	kg
Chloroacetamide	Acetochlor	Warrant	3.8 qts.	2.85	0.52
Acetamide	S-metolachlor	Dual II	1.3 pts.	1.24	0.22
Imidazolinone	Imazapic	Plateau	12 oz. §	0.19	0.03
Aryl triazinone	Carfentrazone	AIM	1.4 oz. §	0.02	0.001
Dinitroaniline	Pendimethalin	Prowl	2 pts. ¶	0.95	0.174
Pyrazole	Pyrasulfotole	Huskie	15 oz. §	0.21	0.038
Isoxazole	Isoxflutole	Balance Pro	3 oz.	0.01	0.001
Substituted urea	Diuron	Direx	4.8 qts. §	4.80	0.881
Triazine	Atrazine	Aatrex	3 pts. ¶	1.50	0.275
Benzoic acid	Dicamba	Banvel	3 pts.	1.50	0.275
Sulfonylurea	Halosulfuron	Permit	2/3 oz.§	0.31	0.056

§ - Non-ionic surfactant (0.25% v/v) ¶ - Crop oil surfactant (1.5% v/v)

VISUAL INJURY SCORING SCALE

Leaf and shoot phytotoxicity will be assessed using a 0-5 scale (Smith et al. 2013)

0 No injury. Plant has no visible injury caused by chemical treatment.

1 Slight injury. Plant has injury spots covering 5% of total observed leaf area.

2 Moderate. Plant has injury covering 5%-25% of total leaf area.

3 Significant. Plant has injury covering 25%-50% of total leaf area.

4 Severe. Plant has injury covering 50%-99% of total leaf area.

5 Death. Plant dies from phytotoxic effect.

GREENHOUSE SCREENING OF POST EMERGENT HERBICIDES

The first greenhouse test (Greenhouse Screen #1) was a randomized complete block designed experiment in which the phytotoxic effects of selected POST herbicides applied over-the-top at the 3-5 leaf stage were observed and scored. Experimental units consisted of nine potted plants, which were treated with selected POST herbicides (Table 1) and one control treatment consisting of water and was replicated four times. Total injury was determined through relative phytotoxicity visual scoring, measuring plant height, and measuring relative value of chlorophyll quantity. A chlorometer (Apogee Instruments, Model CCM-200) was used to measure the relative chlorophyll quantity of the leaves and measurements were recorded. The chlorometer measured the absorbance of bands of light present in the blue and red but not in the green or infrared bands to estimate the chlorophyll quantity in leaf tissue. Using the chlorophyll light absorbance range of 653 nm and a compensation range, for mechanical effect such as tissue thickness, of 931 nm, the chlorometer measures the absorbance of both wavelengths to calculate a Chlorophyll Concentration Index (CCI) value which is proportional to the amount of chlorophyll in the sample.

CCI was measured 24 hours after treatment and then every 7 DAT for 21 days by taking the average of 5 chlorophyll measurements from where the leaf mid-rib ends on uppermost true leaf for 3 random samples treatment^{-1} and PMN leaves were wiped clean of residue paper towel so that chlorophyll quantity could be measured properly prior to first sample. All CCI samples were collected prior to visual injury assessments. Total height plant^{-1} was measured at 2 DAT and then every 7 DAT until 21 days for each plant. Visual injury scores were taken for each plant 24 hours after treatment and then every additional 7 DAT for 21 days.

The second greenhouse test was a randomized complete block designed experiment which phytotoxic effects of selected POST herbicides applied over-the-top at the at the 7-9 leaf stage were observed. Experimental units consisted of 5 plants, which were treated with selected POST herbicides (Table 1) and one control treatment consisting of water and replicated four times. Visual injury scores (1-5) for each individual were taken 24 hours after treatment and then every additional 7 DAT for 21 days. CCI was measured 24 hours after treatment and then every 7 DAT for 21 days by taking the average of 5 chlorophyll measurements from where the leaf mid-rib ends on uppermost true leaf for 3 random samples treatment^{-1} and PMN leaves were wiped clean of residue paper towel so that chlorophyll quantity could be measured properly prior to first sample. All CCI samples were collected prior to visual injury assessments. Total height plant^{-1} was measured at 2 DAT and then every 7 DAT until 21 days for each plant.

For both greenhouse experiments the selected herbicides (Table 1) were sprayed over-the-top of plants with a liquid carbon dioxide pressurized backpack sprayer calibrated at 85 L ha^{-1} using flat fan spray nozzles, at 27 m minute^{-1} and at 172 kilopascal tank pressure. A crop oil surfactant or non-ionic surfactant was used when necessary according to their respective industry labels. Due to the lack of commercial seed production, clonal propagules from a F_1 nursery were used to establish plants into one gallon pots filled with commercial growing soil and placed under artificial light (800 W growing bulbs) with a 13:11 hour light:dark schedule. To prevent drift and facilitate chemical settling, a box-enclosure was used; as well as a metronome in order to keep proper application pace. Irrigation was applied 12 hours after treatments by overhead sprinklers and then for the remainder of the experiment plants were watered by hand-wand until soil saturation.

FIELD SCREENING OF POST EMERGENT HERBICIDES

A replicated field trial to observe phytotoxic effects against selected POST herbicides through the 5-9 leaf stage was initiated in the summer of 2014 at the Texas A&M AgriLife Research Farm in Burleson, TX (N30.5474, W96.4357). During the winter of 2013, PMN seed were increased by the Perennial Grass Breeding and Genetics Laboratory at Texas A&M. A total of 57 plots of PMN were established into a well prepared seed bed on July 7, 2014 into a fine-silty, mixed, superactive, thermic Udifluventic Haplustepts. (Westwood series) soil by a tractor with a 3-point gear-driven JP-H planter. The soil was vertically disked, tilled, and firmed by heavy roller prior to planting. Each plot measured 3.04 x 1.21 m and planted as twin rows (38 cm separation), with 1.21 m between ranges and 1.5 m between rows. Seeds were planted 7.62 cm apart and 2 seed hole^{-1} for a total of 80 seeds per twin-row plot. Upon establishment (3-5 true leaves) plots where thinned to 40 plants total. At 5-9 true leaves, selected POST herbicides (Table 1), applied alone and in various labeled combinations, were sprayed over-the-top of plants employing a CO_2-pressurized 3-point sprayer at 85 L ha^{-1} using a flat fan spray nozzle, at 27 m minute^{-1} and at 172 kPa tank pressure. A crop oil surfactant or non-ionic surfactant was used when necessary according to industry label.

The experiment was arranged in a randomized complete block design with 19 treatments (including a non-treated control) and three replications. Plants were harvested on October 18, 2014. In addition to injury scores and relative CCI, total yield, average dry matter, and plant height were used to assess phytotoxicity. A chlorometer (Apogee Instruments, Model CCM-200) was used to measure relative CCI of the leaves. Measurements were made immediately prior to visual assessments by taking the average of 5 chlorophyll samples from the most upper true leaf for 3 random samples treatment^{-1}. CCI was measured 24 hours after treatment and every 7 DAT for 42 days. Irrigation was applied 12 hours after treatments by in-field sprinklers, then by flood irrigation for the remaining duration. Each research plot was kept weed free throughout the experiment by hand pulling and hoeing the weeds to eliminate inaccurate injury ratings caused by dead / dying weeds and local competition. Sampled leaves were wiped clean of residue a with wet paper towel so that CCI could be measured properly.

ERADICATION

During the fall of 2014, a test was conducted to assess the eradication efficacy of glyphosate (RoundUp Max®) at three different rates and two different time periods on the PMN hybrids. Twelve plots were randomly selected from the 69 total plots established before the 2014 field test, each plot was 3.04 m x 1.21 m in size and planted as twin rows (38 cm separation), with 1.21 m between ranges and 1.5 m between rows. Seeds were planted 7.62 cm apart within each row and 1to 3 seed were planted hole^{-1}. The two rates evaluated were 0.05 kg hectare^{-1} (4 ounces' acre^{-1}) and 0.14 kg hectare^{-1}

(12 oz. ac^{-1}) of glyphosate. For each treatment, one gallon of solution was sprayed evenly across green tissue with hand-pump back pack sprayer until no water was remaining. Two application time periods of RoundUp were tested: 1) during late fall but before first frost date of November 17 and 2) after last frost date of March 2 but before late spring.

GREENHOUSE SCREENING OF PRE-EMERGENT HERBICIDES AND RESPECTIVE SEED SAFENER

During the spring of 2015, greenhouse tests were performed to observe phytotoxic effects of selected seed safeners through dose response. The experimental design was a randomized complete block with 3 replications. Experimental units consisted of 10 PMN seeds planted into flats of 25-count 9 in deep-well containers (5 wells row^{-1}) filled with a fine-silty, mixed, superactive, thermic Udifluventic Haplustepts. (Westwood series) soil. Treatment wells were randomly assigned by random number generator (1-6) with 2 treatments $tray^{-1}$. Selected safeners (Table 6) were tested against companion herbicide alone at the same application rates used in the field study (Table 1) and at 6 different rates of g active ingredient kg^{-1} of safener to seed, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00. Stock solutions made by mixing a stock solution of 1 g of a.i. with 10 ml of distilled water and then adding 2.5% surfactant sodium dodecyl sulfate, mixing for 24 hours with plate stirrer. Seed treated with prepared aqueous solutions of seed safener prior to seeding and left to air dry for 1 hour at 27º C. To ensure proper planting depth, a galvanized steel punch marked at 6.5 mm was used in each cell container. Initial irrigation commenced 1 hour after treatment and was applied by squeeze bottle until soil saturation. For the duration of experiment, flats were watered by low-pressure hand-wand three times weekly until soil saturation. Phytotoxicity was assessed by counting seed emergence treatment^{-1} each day for 14 days.

STATSTICAL ANALYSIS

All data were subjected to analysis of variance (ANOVA) using SPSS 11.0 for Windows 8. Means separated using Fisher's protected least significant difference test, significance level set at 0.05 and 0.01 (SAS 2007).

CHAPTER IV

RESULTS AND DISCUSSION

GREENHOUSE SCREEN #1 OF POST EMERGENT HERBICIDES

The experiment was replicated 2 times in sequence starting on Nov 7, 2013 - Nov 28, 2013 and then again on Dec 10, 2013 – Jan 10, 2014. Based on material safety data sheets and specimen label, herbicide injury due to phytotoxicity (Table 2) were mostly as expected. Significant phytotoxic injury was found for several of the herbicides tested. Due to the small width of the leaf blades, CCI could not be measured accurately enough to be included for screen #1. Observed significant effects of each herbicide are outlined below.

Permit (halosulfuron), Prowl (pendimethalin), Banvel (dicamba), and AIM (carfentrazone-ethyl) treated plants resulted in no significant changes in injury or plant height.

Plateau (imazapic) treated plants resulted in significantly higher injury scores and shorter plant heights at 21 DAT as compared to non-treated plants.

Aatrex (atrazine) treatments resulted in significantly shorter plant height and higher injury scores compared to the non-treated plants.

Direx (diuron), had significantly lower plant height and higher injury scores as compared to the non-treated plants. This herbicide group killed all plants within 21 days. It is important to note that the rate used for this herbicide was a non-crop rate and, as expected, it significantly injured the PMN plants.

Warrant (acetochlor) resulted in significantly shorter plant height as compared to the non-treated plants; however, injury scores were not significant.

Huskie (pyrasulfotole) treated plants had significantly shorter plant height as compared to the non-treated plants; however, injury scores were not significant.

GREENHOUSE SCREEN #2 OF POST EMERGENT HERBICIDES

This experiment was replicated 2 times: Dec 20, 2013- Jan 10, 2014; and Jan 13, 2014 – Jan 24, 2014. Herbicides were categorized as safe, partially safe, and unsafe based on results from ANOVA on two metrics: CCI and injury scores. Plant height was not considered in categorizing

safety due to the lack of any significant changes in potted plant heights at this stage of growth. Based on material safety data sheets and specimen label, herbicide injury due to phytotoxicity (Table 3) were mostly as expected and significant phytotoxic injury was found in several of the herbicides tested. Observed significant effects of each herbicide are outlined below.

Plateau (imazapic) treated plants began significant visual decline in plant health around 17 DAT, and these effects worsened as time elapsed beyond the test. Significantly lower CCI was measured 21 DAT but injury scores and plant heights were not different.

Permit (halosulfuron) performed as expected with no significant changes in CCI, injury, or plant height.

Table 2. Greenhouse screen of selected herbicides at the 3-5 leaf stage, mean injury for all weeks and plant heights until 21 DAT.

Treatment	Rate	Injury	Height
	lb a. i. $acre^{-1}$	Score (1-5)	cm
Non-treated		1 a	38 a
Huskie	0.21	2 a	32 a
Prowl	0.95	2 a	30 a
Plateau	0.19	3 b	20 b
Permit	0.31	1 a	35 a
Direx	4.8	4 b	--
Banvel	1.5	2 a	40 a
AIM	0.02	1 a	35 a
Aatrex	1.5	3 b	27 b
Warrant	2.85	2 a	27 b
P < F (ANOVA)		< 0.05	< 0.05
Total Samples (n)		720	72

Means within columns followed by a common letter are not significantly different (LSD, $P = 0.01$ and 0.05).

Prowl (pendimethalin) treated plants had significantly lower CCI at 7, 14, and 21 DAT content as compared to the non-treated plants; however, when these plants were compared to the non-treated plants, injury scores and plant height were not different.

Banvel (dicamba) treated plants resulted in significantly lower CCI at 7 and 14 DAT as compared to the non-treated plants; however, this herbicide did not significantly injury the plant visually.

Aatrex (atrazine) treatments resulted in significantly lower plant height and higher injury scores compared to the non-treated plants and CCI was lower at 14 DAT was significantly lower.

Table 3. Second greenhouse screen at 7-9 leaf stage mean CCI and injury scores for all weeks until 21 DAT. Relative safety of herbicide is listed under category.

Treatment	Rate	Relative chlorophyll count				Injury	Plant height	Category
	lbs. a. i. acre^{-1}	1	7	14	21	Score (1-5)	cm	
Non-treated		4.7 a	3.56 a	4.79 a	7.65 a	1 a	53 a	
Huskie	0.21	6.9 a	2.36 b	2.88 b	5.77 b	2 a	50 a	Safe
Prowl	0.95	4.5 a	2.87 b	3.43 b	4.16 b	2 a	52 a	Safe
Plateau	0.19	7.1 a	3.73 a	4.68 a	5.91 b	2 a	48 a	Safe
Permit	0.31	5.2 a	3.64 a	4.84 a	7.23 a	1 a	50 a	Safe
Direx	4.8	4.4 a	1.97 b	1.12 b	1.35 b	4 b	48 a	Un-Safe
Banvel	1.5	3.8 a	2.98 b	3.65 b	6.57 a	2 a	53 a	Safe
AIM	0.02	4.2 a	3.25 a	4.90 a	7.85 a	1 a	50 a	Safe
Aatrex	1.5	5.8 a	3.38 a	4.06 b	6.86 a	2 a	48 a	Safe
Warrant	2.85	3.4 a	3.17 a	4.01 b	6.01 a	2 a	53 a	Safe
P < F (ANOVA)		----	< 0.01	< 0.01	< 0.01	<0.05	<0.01	
Total Samples (n)		960	960	960	960	800	72	

Means within columns followed by a common letter are not significantly different (LSD, P = 0.01 and 0.05).

Direx (diuron) treated plants resulted in plant death for 75% of the plants by the end of both greenhouse experiments. Treated plants had reduced CCI at 7, 14, and 21 DAT and mean injury scores were significantly higher as compared to the non-treated plants.

AIM (carfentrazone-ethyl) behaved as expected with no significant changes in CCI, injury, or plant height.

Warrant (acetochlor) treated plants had significantly lower CCI at 14 DAT; however, injury scores and plant height were not statistically different.

Huskie (pyrasulfotole) treatments resulted in significantly lower CCI at 7, 14, and 21 DAT; however, injury scores and plant height were not statistically different.

FIELD SCREENING OF POST EMERGENT HERBICIDES

Significant phytotoxic injuries, differences in plant height, yield reduction, and injury scores were measured for several of the herbicides tested (Tables 4 and 5). Overall, the herbicides behaved as expected and according to their respective labels, they injured the vigorous PMN plants which eventually outgrew all injury during the short harvest. Significant effects of each herbicide are outlined below by herbicide group.

Plateau (imazapic) applied alone had significantly lower CCI at 21, 28, 32 DAT. When mixed with AIM it had lower CCI at 28, 35, and 42 DAT and when mixed with Direx CCI was significantly lower at 21, 28, 35, and 42 DAT. Injury scores, plant height and total yield were significantly different when used alone and in combination with AIM and with Direx.

Permit (halosulfuron) alone had significantly lower CCI at 42 DAT as compared to the non-treated plants and when mixed with Huskie the CCI was significantly lower at 7 DAT. Plant height and total yield were significantly different as compared to the non-treated plants when used alone, and in combination with Huskie and Dicamba.

Prowl (pendimethalin) treated plants had significantly lower CCI at 14 DAT, and were shorter in average plant height. When mixed with Warrant, CCI was significantly lower at 14 DAT.

Banvel (dicamba) when applied alone significantly lowered CCI at 42 DAT, and these plants where shorter in plant height and yielded less as compared to the non-treated plants. This treatment did not result in significant injury. When mixed with Permit, there were significant differences in yield and plant height but injury was not significant.

Table 4 – Field screen at 5-9 leaf stage, CCI means from 7-42 DAT

Treatment	Rate	Relative chlorophyll count					
	lbs a. i. acre^{-}	7	14	21	28	35	42
Non-treated		11.18 a	12.41 a	17.37 a	17.40 a	17.88 a	23.57 a
Huskie	0.21	14.24 b	15.34 a	13.03 b	15.38 a	20.05 a	18.82 b
Prowl + Warrant	0.95 + 2.85	13.66 a	9.13 c	17.12 a	14.46 a	16.96 a	24.51 a
Prowl	0.95	8.32 a	15.36 b	17.47 a	16.14 a	15.06 a	19.38 a
Plateau + Direx	0.19 + 4.8	13.13 a	12.79 a	8.17 b	12.07 b	11.01 b	17.34 b
Plateau + AIM	0.19 + 0.02	7.56 c	12.07 a	19.45 a	13.09 b	11.08 b	18.49 b
Plateau	0.19	8.56 a	11.02 a	11.41 b	13.59 b	12.54 b	21.03 a
Permit + Huskie	0.31 + 0.21	18.34 b	15.12 a	14.55 a	16.34 a	19.52 a	22.53 a
Permit + Banvel	0.31	13.52 a	12.99 a	18.41 a	14.65 a	16.33 a	20.24 a
Permit	0.31	9.02 a	12.46 a	19.42 a	16.16 a	18.97 a	18.61 b
Direx + AIM	4.8 + 0.02	10.26 a	11.71 a	8.87 b	10.47 b	15.10 a	19.02 b
Direx + Warrant	4.8 + 2.85	15.06 b	9.11 c	10.91 b	8.81 b	13.19 b	18.94 b
Direx	4.8	13.72 a	8.86 c	14.19 a	10.64 b	13.71 b	17.86 b
Banvel	1.5	14.81 a	12.34 a	16.13 a	15.31 a	20.53 a	18.45 b
AIM	0.02	19.08 b	15.49 b	11.63 b	14.92 a	16.01 a	18.48 b
Aatrex + Huskie	1.5 + 0.21	14.06 a	11.71 a	13.16 b	11.26 b	16.93 a	21.33 a
Aatrex + AIM	1.5 + 0.02	20.82 b	19.59 b	15.11 a	16.97 a	18.53 a	16.81 b
Aatrex	1.5	8.77 a	14.97 a	10.14 b	13.88 b	13.00 b	21.19 a
Warrant	2.85	14.14 a	11.79 a	9.98 b	14.30 a	14.61 a	18.29 b
P < F (ANOVA)		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	<0.057
Total Samples		171	171	171	171	171	171

Means within columns followed by a common letter are not significantly different (LSD, P = 0.01 and 0.05).

Table 5 – Field screen: yield, final average plant height, and mean injury for herbicides applied post crop emergence onto foliage.

Treatment	Yield	Height	Injury	Category
	kg / plot	cm	score	
Non-treated	26 a	69 a	1 a	
Huskie	20 a	61 b	1 a	Safe
Prowl + Warrant	28 a	75 a	1 a	Safe
Prowl	20 a	59 b	2 bc	Partially safe
Plateau + Direx	11 b	52 b	3 c	Unsafe
Plateau + Aim	11 b	45 b	3 c	Unsafe
Plateau	18 b	61 b	2 bc	Unsafe
Permit + Huskie	13 b	54 b	1 a	Partially safe
Permit + Banvel	12 b	52 b	1 a	Partially safe
Permit	16 b	44 b	1 a	Partially safe
Direx + AIM	16 b	52 b	2 bc	Unsafe
Direx + Warrant	18 b	34 b	3 c	Unsafe
Direx	6 c	34 b	2 bc	Unsafe
Banvel	13 b	53 b	1 a	Partially safe
AIM	18 b	64 a	1 a	Safe
Aatrex + Huskie	27 a	71 a	1 a	Safe
Aatrex + AIM	17 b	59 b	2 bc	Unsafe
Aatrex	21 a	74 a	2 bc	Partially safe
Warrant	21 a	56 b	1 a	Safe
P < F (ANOVA)	< 0.05	<0.05	<0.01	
Total Samples (n)	57	570	855	

Means within columns followed by a common letter are not significantly different.

Aatrex (atrazine) alone showed significantly lower CCI at 21, 28, and 35 DAT and higher injury scores however, did not result in shorter plant height or lower yield as compared to the non-treated plants. When mixed with AIM, CCI was significantly higher at 7 and 14 DAT but lower at 42 DAT with significantly shorter plant height, lower yield, and higher injury scores. When mixed with Huskie, CCI was significantly lower at 21 and 28 DAT, with injury scores being significantly higher than the non-treated plants.

Direx (diuron) treated plants had significantly lower CCI at 14, 28, 35, and 42 DAT, and were shorter in plant height, with lower yields, and higher injury scores as compared to the non-treated plants. When mixed with AIM, CCI was lower at 21, 28, and 42 DAT, with shorter plant height, lower yield, and higher plant injury scores. When mixed with Warrant, CCI was significantly lower 7, 14, 21, 28, 35, and 42 DAT, with significantly shorter plant height, lower yield, and higher injury scores.

GREENHOUSE SCREEN OF SEED SAFENER

Experiment was replicated 1 time on March 2, 2015 - March 16, 2015. At various safener rates applied as in-tank combination with paired herbicide (Table 6), germination was significantly reduced due to phytotoxic response from herbicides as compared to the non-treated plants. Safeners were categorized as safe and not safe; based on the higher two rates combined with significance from ANOVA. Considered safe at $\geq .75$ g kg^{-1} of a.i are: NA + Plateau, Flouxofenim + Dual II, Cyprosulfamide + Balance Pro, and Isoxadifen + Permit. The combination Flouxofenim + Dual II had significantly much lower germination rates with lower safener rates. There were no significant variations in germination of PMN at .75 and 1 g kg^{-1} of seed for each safener / herbicide combination.

ERADICATION OF TEST PLOTS

Experiment was initiated on Nov 10, 2014. Findings from the experiment revealed that all rates of herbicides applied were successful at eradicating the plants in all of the test plots. Because of the successful eradication of plants in the fall, there was no need for a

Table 6 – Greenhouse screen of selected safeners, mean germination rates.

Treatment	% safener rate (g kg^{-1} of a.i applied to seed)						Category
	0.05	0.10	0.25	0.50	0.75	1	
	Germination % mean scores						
Non-treated	93 a	97 a	90 a	97 a	90 a	93 a	
Naphthalic Anhydride + Plateau	48 b	51 b	88 a	88 a	88 a	90 a	Partially safe
Flouxofenim + Dual II	55 b	63 b	63 b	84 b	89 a	90 a	Partially safe
Isoxadifen + Permit	90 a	89 a	87 b	88 a	90 a	88 a	Safe
Cyprosulfamide + Balance Pro	90 a	87 a	93 a	87 b	90 a	89 a	Safe
P < F (ANOVA)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Total samples (n)	45	45	45	45	45	45	

Means within columns followed by a common letter are not significantly different (LSD, *P* = 0.01)

spring treatment. The time of herbicide application of herbicide coincided with the natural withdraw of nutrients towards the roots of the plant due to winter dormancy.

CHAPTER V

CONCLUSIONS

Several herbicides were identified that are useful for the safe establishment of seeded PMN (Table 7). Based on analysis of dose response we found the use of the pre-crop emergent herbicides Balance Pro, Dual II, Plateau, and Permit, to be safe; so long as appropriate rates of seed safener were used. Based on analysis of three dose response tests, the post-crop emergent herbicides Warrant, AIM, Aatrex, Banvel, Permit, Huskie, and Prowl are generally safe for use when applied to foliage of field-established PMN plants after the 5th leaf stage when used at similar rates observed here. When timed appropriately, or in situations where weed control is unexpectedly necessary, these herbicides can be considered generally safe to use even though some of the herbicides resulted in significantly reduced overall yield and/or caused slight injury during a short growing season (103 days). This is because these herbicides did not result in plant death in the tests performed but should have resulted in weed death according to commercial herbicide label. In contrast, Direx and Plateau resulted in plant death when applied at the 3-7 leaf stage during the greenhouse screens for all treated plants and some individual plant death respectively. Direx alone caused significant plant death when applied at the 7-9 leaf stage during the field study; however, the rate selected was for non-crop eradication (4.5 L) and the typical row-crop rates for commercial Direx applications are much lower (0.7 L- 1 L) and should produce significantly less damage to establishing plants. Also, we tested these herbicides over-the-top and the Direx label recommends directed spray inter-row in order to target weeds and reduce non-target leaf surface contact on row crops such as cotton, corn, and sugarcane. If used appropriately and according to label, including the recommended field test of over-the-top and pre-emergent applications to a small area, each the herbicides tested here can successfully be used to kill competing weeds in PMN stands during establishment and beyond.

Table 7 - Commercial herbicides established by this study as generally safe for use during establishment of PMN by seed

Herbicide	Labeled rate $acre^{-1}$	Active ingredient $g\ kg^{-1}$	Crop emergence timing	Weed emergence timing	Application method **	Kills *	Crop label *
AIM	1.4 oz. §	0.02	PRE-PLANT, PRE, POST	POST	F	D	M
Aatrex	3 pts. ¶	1.5	PRE-PLANT, PRE, POST	PRE, POST	I F S	M D	M
Balance Pro ¥	3 oz.	0.01	PRE	PRE	I S	D	M
Banvel ¥	3 pts.	1.5	PRE-PLANT, POST	POST	F	D	M
Dual II ¥	1.3 pts.	1.24	PRE-PLANT, PRE	PRE	I F S	M D C	M D
Huskie	15 oz. §	0.21	PRE-PLANT, POST	POST	F	D	M
Permit	2/3 oz. §	0.31	PRE, POST	PRE	I S	D	M D
Prowl	2 pts. ¶	0.95	POST	PRE	I S	M D	M D
Warrant ¥	3.8 qts.	2.85	PRE, POST	PRE	I S	M D	M

¥ Use this herbicide with appropriate safener

REFERENCES

Aganga, A.A., U.J. Omphile, T. Thema, and J.C. Baitshotlhi. 2005. Chemical composition of napier grass (*Pennisetum purpureum*) at different stages of growth and napier grass silages with additives. J. Biol. Sci. 5: 493-496.

Andrews, D.J., J.F. Rajewski, and K.A. Kumar. 1993. Pearl millet: New feed grain crop. p. 198-208. In: J. Janick and J.E. Simon (eds.), New crops. Wiley, New York.

Babcock, B. 2010. Opportunity for profitable investments in cellulosic biofuels. Energy Policy. 39: 714-719.

Bains, K. 1971. Genetic analyses for certain plant and ear characters in pearl millet top-crosses. Theor. Appl. Genet. 41: 302-305.

Bora, S., R. Bhuyan, D.N. Sarma, K.K. Sharma, and A. Bora. 2012. Effect of Variety and Stage of Harvest on the Yield, Chemical Composition and In-vitro Digestibility of Hybrid Napier (Pennisetum purpureum × P. americanum). Indian J. Anim. Nutr. 28:418-420.

Brunken, J. 1977. A Systematic Study of Pennisetum sect. Pennisetum (Gramineae). Am. J. Bot. 64:161-176.

Burton, G.W. 1944. Hybrids between Napiergrass and Cattail millet. J. Herd. 35:227-232.

Butler, T.J., and J.P. Muir. 2006. Coastal Bermudagrass (*Cynodon dactylon*) Yield Response to Various Herbicides. Weed Tech. 20:95-100.

Caffrey, K R. 2014. The farm to biorefinery continuum: A techno-economic and lca analysis of ethanol production from sweet sorghum juice. Agric. Syst. 130, 55-66.

Carson, A.G. 1987. Improving weed management in the draft animal-based production of early pearl millet in The Gambia. Trop. Pes. Manage. 33:359-363.

Chemisquy, M.A., L.M. Giussani, M.A. Scataglini, E.A. Kellogg, and O. Morrone. 2010. Phylogenetic studies favour the unification of Pennisetum, Cenchrus and Odontelytrum (Poaceae): a combined nuclear, plastid and morphological analysis, and nomenclatural combinations in Cenchrus. Ann. Bot. 106:107-130.

Chikafumbwa, F.J.K. 1996. The use of Napiergrass (*Pennisetum purpureum*) and maize (*Zea mays*) bran as low-cost tilapia aquaculture inputs. Aquaculture 146:101-107.

Correll, D., Y. Suzuki, and B.J. Martens. 2014. Logistical supply chain design for bioeconomy applications. Biomass Bioenergy. 66:60-69.

Dan, H.A. 2010. Seletividade do herbicida tembotrione à cultura do milheto. Selectivity of the herbicide tembotrione to pearl millet. Planta Daninha 28:793.

EISA. 2007. Energy Independence and Security Act of 2007, Pub. L. No. 110-140, 121 Stat. 1492, 1783-84 (Dec. 19, 2007), codified at 42 U.S.C. §17381.

Environmental Protection Association. 2015. Final Renewable Fuel Standards for 2014, 2015 and 2016, and the Biomass-Based Diesel Volume for 2017. https://www.epa.gov/renewable-fuel-standard-program/final-renewable-fuel-standards-2014-2015-and-2016-and-biomass-based#rule-summary

Farinelli, R. 2005. Efficiency of Herbicide 2,4 D in the control of *Raphanus raphanistrum* L., after-emergence of Pearl Millet. Revista Brasileira Milho Sorgo 4: 104.

Fedorov, A.A. (Ed). 1969. Chromosome numbers of flowering plants. Academy of Sciences of the USSR, V.L. Komarov Botanical Institute, Nauka, Leningrad

Gelfand, I. 2013. Sustainable bioenergy production from marginal lands in the US Midwest. Nature 493:514-517.

Gould, F., and R. Shaw. 1983, Grass Systematics. 2nd ed. Texas A&M Univ. Press, College Station.

Grichar, W.J. 2008. Weed Control and Bermudagrass Tolerance to Imazapic Plus 2,4-D. Weed Tech. 22:97-100.

Gulia, S.K., J.P. Wilson, B.P. Singh, J. Carter. 2006. Progress in grain pearl millet research and market developments. 6th New Crops Symposium, Assoc. Adv. of Industrial Crops.

Gwayumba, W., D.A. Christensen, J.J. McKinnon, and P. Yu. 2002. Dry matter intake, digestibility and milk yield by Friesian cows fed two Napier grass varieties. Asian-Aust. J. Anim. Sci., 15:516-521.

Habiyaremye, C., J.B. Matanguihan, Guedes J. D'Alpoim, et al. 2016. Proso Millet (Panicum miliaceum L.) and Its Potential for Cultivation in the Pacific Northwest, U.S.: A Review. Front. Plant Sci. 7:1961.

Hanna, W. W., and W.G. Monson. 1980. Yield, Quality, and Breeding Behavior of Pearl Millet × Napiergrass Inter-specific Hybrids. Agron. J. 72-360.

Hettinga, W.G. 2009. Understanding the reductions in US corn ethanol production costs: An experience curve approach. Energy Policy. 37:190-203.

Hopkinson, C.S. 1980. Net energy analysis of alcohol production from sugarcane. Science. 207:302-304.

IPCC. 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jessup, R.W. 2013. Seeded-Yet-Sterile' Perennial Biofuel Feedstocks. Adv. Crop Sci. Tech. 1:e102.

Kering, M.K., C. Huo, S.M. Interrante, D.W. Hancock, and T.J. Butler. 2013. Effect of Various Herbicides on Warm-season Grass Weeds and Switchgrass Establishment. Crop Sci. 53:666-673.

Khairwal, I.S., K.N. Rai, B. Diwakar, Y.K. Sharma, B.S. Rajpurohit, B. Nirwan, and R. Bhattacharjee. 2007. Pearl Millet Crop Management and Seed Production Manual. Manual. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.

Knoll, J. 2012. Vegetative Propagation of Napiergrass and Energycane for Biomass Production in the Southeastern United States. Agron. J. 104:518-522.

Ndahi, W.B. 1981. Pearl Millet Tolerance to Selected Herbicides. Trans. Kansas Acad. Sci. 84:105-108.

Ndahi, W.B. 1984. Herbicide evaluation in millet in the northern Guinea and Sudan savannah of Nigeria. Trop. Pest Manage. 30:351-355.

Nelson, C.J. and L.E. Moser. 1994. Plant factors affecting forage quality. (editor) G.C. Fahey, Jr. USA, p. 115-154 *in* Forage Quality, Evaluation, and Utilization. Asa, Cssa, and Sssa, Madison, WI.

OMM. 1993. L agrometeorologie du mil (*Pennisetum glaucoma* (L) Leeke). Rapport CMAg No 56.

Orodho, A.B. 1990. Utilization of research results on forages and agricultural by-products materials as animal feed resources in Africa. Proceedings of the first joint workshop held in Lilongwe, Malawi 5-9 December 1998 by Pasture Network for Eastern and Southern Africa (PANESA) and African Research Network for Agricultural By-products (ARNAB).

Pacheco, L.P., F.A. Petter, A.C.F. Câmara, D.B.C. Lima, S.O. Procópio, A.L.L. Barroso, et al. 2007. Tolerância do milheto (*Pennisetum americanum*) ao 2, 4-D. Planta Daninha. 25:173-179.

Robinson, D.E., N. Soltani, C. Shropshire, and P.H. Sikkema. 2013. Cyprosulfamide Safens Isoxaflutole in Sweet Corn (*Zea mays* L.). Hort. Science. 48:1262-1265.

Rubin, E. 2008. Genomics of cellulosic biofuels. Nature. 454:841-845.

Shaner, D.L. 2014. Herbicide handbook of the Weed Science Society of America. 5th ed. Herbicide Handbook Committee.

Smith J., B. Wherley, P. Baumann, S. Senseman, R. White, et al. 2013. Early Summer Slender Aster Control in Bermudagrass using Bioherbicide *Phoma macrostoma*. J. Biofertil. Biopestici. 4:139-143.

Soreng, R. J., P. M. Peterson, K. Romaschenko, G. Davidse, F. O. Zuloaga, E. J. Judziewicz, T. S. Filgueiras, Davis, and O. Morrone. 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). J. Syst. Evol. 53:117-137.

Staal, S.J., R. Kruska, J. Balteweck, M. Kenyanjui, A. Wokobi, D. Njubi, P. Thornton, and W. Thorpe. 1999. Combined household and GIS analysis of smallholder production systems. An application to intensifying smallholder dairy systems approaches for Agricultural Development (SAAD-III) held in Lima, Peru, 8-10 November 1999. National Agrarian University, La Molina, Lima, Peru.

Stahlman, P., W., P. W. Geier, and L. D. Charvat. 2009. Tolerance of three millet types to saflufenacil. Proc. North Centr. Weed Sci. Soc. 64:127-134.

Stapf, O., and C.E. Hubbard. 1934. Pennisetum. Flora of Tropical Africa. p. 954–1070. *In*: Prain D, (editor). Ashford: L. Reeve & Co. Ltd.

Tiwari, U.P. 2014. Nutritional characteristics and digestibility by near-infrared spectroscopy of local and hybrid napiergrass varieties grown in rain-fed and irrigated conditions. Anim. Prod. Sci. 54: 1775-1778.

Turhollow, A. 1994. The economics of energy crop production. Biomass Bioenergy 6:229-241.

Verma M., S. Godbout, S.K. Brar, O. Solomatnikova, S.P. Lemay, and J.P. Larouche. 2012. Biofuels Production from Biomass by Thermochemical Conversion Technologies. Int. J. Chem. Eng.

Wright, L.L. 1994. Production technology status of woody and herbaceous crops. Biomass Bioenergy. 6:191-209.

Yates, N.G., and T. Pangabean. 1988. The performance of goats offered elephant grass (*Pennisetum purpureum*) with varied amounts of *Leucaena leucacephala*. Trop. Grassl. 22:126-131.

Zeri, M., M.Z. Hussain, K.J. Anderson-Teixeira, E. DeLucia, and C.J. Bernacchi. 2013. Water use efficiency of perennial and annual bioenergy crops in central Illinois. J. Geophys. Res.: Biogeosciences. 118:581-589.