8-9-2022

Phenotypic evaluation of energycane (Saccharum spp.) genotypes in northcentral Mississippi

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agronomy
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

August 2022

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Date of Degree: August 9, 2022

Institution: Mississippi State University

Major Field: Agronomy

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Title of Study: Phenotypic evaluation of energycane (*Saccharum* spp.) genotypes in northcentral Mississippi

Pages in Study: 106

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As fossil fuel supplies decrease and concerns of climate change increase, the search for alternative sources of fuel has pushed biomass crops to the forefront of discussion. *Saccharum spontaneum* readily hybridizes with commercial sugarcane and lends cold tolerance and greater yields to the hybrid progeny, called energycane. Twenty genotypes were tested against an energycane variety (Ho 02-113) as a control. Two locations were tested: the HH Leveck Animal Research Center (planted in 2019), and the Bearden Dairy Research Center (planted in 2020). The Bearden Dairy Research Center yielded significantly higher than the HH Leveck Animal Research Center regarding dry matter yield ($P < 0.0001$). Dry Matter yields were significantly greater in the plant cane year than in the ratoon year at the HH Leveck Animal Research Center ($P = 0.0008$). There were significant differences among replications in both locations regarding dry matter.

ACKNOWLEDGEMENTS

This work was funded by the DOE Center for Advanced Bioenergy and Bioproducts Innovation (U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018420). Any opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of the U.S. Department of Energy. This work was completed jointly with DOE Center for Advanced Bioenergy and Bioproducts Innovation, and Mississippi State University.

TABLE OF CONTENTS

APPENDIX

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND OBJECTIVES

Introduction

The demand for energy is growing constantly. Fossil fuels accounted for about 84% of global primary energy and produced about 64% of electricity in 2019 (Ritchie & Roser, 2019). Concerns about a shrinking supply of petroleum, coupled with its negative environmental impacts, have pushed biofuels to the front of the energy discussion. Biofuels offer a renewable source of energy, as well as a potential net reduction in carbon emissions. Among the frontrunners for biofuels are perennial grasses. These represent renewable biomass with reduced inputs compared to annual crops, which require annual planting, and pesticide and herbicide application. Perennial grasses can be harvested and re-emerge the following season from the crown. Sugarcane (*Saccharum* spp.) has potential as a bioenergy crop, but its tropical origin limits northern production due to a lack of cold tolerance. Research on early generation hybrids between sugarcane and *S. spontaneum* began in the 1960's. The sugarcane industry in Louisiana was failing due to sorghum mosaic virus. The Himalayan *S. spontaneum* genotype US 56-15-8 was introduced into the breeding program because it showed resistance to the virus. These hybrids were backcrossed to sugarcane; energy production was not yet the intent of this introduction (Hale, personal communication).

Sugarcane is a large, tropical grass native to Southeast Asia. Sucrose is stored in the pith tissue of the canes (botanically stalks). Energycane has less sugar and greater fiber than

sugarcane, making it economically unsuitable as a sugar crop in most locations but valuable as a biomass crop. High-fiber genotypes that were unsuitable for commercial sugar production found new uses during the “oil shocks” in the 1970s. A renewed interest in energycane breeding followed in the 1990s, specifically selecting for cold hardiness. Breeding energycane for biotic and abiotic stresses could greatly expand its production into marginal lands and more temperate regions, as well as lengthen the growing season.

Sugarcane yields in Louisiana achieve 94 Mg ha^{-1} gross cane weight and 23 Mg ha^{-1} dry weight. This far exceeds other biomass crops being tested in the United States. Energycane production in Louisiana already exceeds 100 Mg ha^{-1} gross cane weight. Energycane could well become the next major bioenergy crop in the United States; however, more testing is needed (Schmitz et al., 2020; Aragon et al., 2017). In 2007, 11 genotypes were tested in Starkville, Mississippi (33.45º N) for winter hardiness. Two years later, 478 genotypes were planted as seedlings in a freeze-tolerance experiment. Screening temperatures didn’t occur until 2011 with temperatures falling below freezing (-9 to -4 °C) for seven days. Only 17 genotypes survived the low winter temperatures, indicating true genetic differences among the progeny and the potential for further selection (Baldwin, personal communication).

Energycane has the potential to diversify agriculture and reduce petroleum fuel needs in the United States. Energycane is estimated to have a greater net energy ratio than elite sugarcane varieties. Germplasm from crosses between commercial sugarcane and wild *S. spontaneum* is usually associated with a lesser sucrose yield and greater fiber content. However, it is also associated with a greater biomass yield per unit land, an increased resistance to disease, and cold weather tolerance. This affords energycane a greater potential climate range than current commercial sugarcane cultivars. Its genetic diversity, cellulosic ethanol production

potential, and ability to ratoon with consistent yields for up to five years with minimal nutrient requirements make it a candidate as a biomass crop for bioenergy. Breeding and variety testing are necessary to determine its geographic limitations. This research gathered morphometric data such as trans-seasonal growth, yield, and stand density as well as chemical composition (soluble sugars, fiber content, and composition) in order to aid in selection of genotypes suitable for production at 33°N latitude and determine genotypes that may give progeny with cold tolerance.

Objectives

The aim of this two-year study is to evaluate 20 energycane genotypes against the control variety (Ho02-113) to determine suitability to latitude 33°N.

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CHAPTER II

REVIEW OF LITERATURE

Bioenergy

Past and Present

Fossil fuels' negative impact on the environment and shrinking supply are two concerns facing energy production today. According to a study on fuel by British Petroleum in 2016, the world has about 50 years' worth of crude oil remaining (BP Statistical Review of World Energy, 2016). In 2014, the United States was the world's largest producer of oil (Ritchie & Roser, 2017). The United States was also the largest single consumer of oil in 2018 (U.S. Energy Information Administration, 2020). In 2019, petroleum accounted for about 33% of primary fuel consumption world-wide (BP Statistical Review of World Energy, 2020). However, our reliance on fossil fuels may be slowing. Renewable energy (including biofuels) grew by the largest increment for any source of energy in 2019. The United States was the second largest contributor to the growth in renewables in 2019 (British Petroleum, 2020).

Biomass is a renewable energy resource that is derived from living or recently living organic matter (Ciolkosz, 2009). It includes food crops, forest residue, agricultural residue, purpose-grown grasses, microalgae, and other resources. Plants capture energy from the sun and store it in the form of chemical bonds (sugars, starches, cellulose, and lignin) that can later be converted to fuel. The products of these catabolic processes are various liquid fuels and thermal energy. Biofuels offer a stable and renewable resource to buffer volatile crude oil prices, supply

clean energy, and generate jobs in rural communities (Rogers et al., 2016). Biofuel is not a new concept. In 1900, Rudolf Diesel debuted his engine at the Paris World's Fair that ran on peanut oil. Diesel said in a newspaper reflecting on the event, that motor-power can be produced from the energy from the sun, which will always be available, whereas solid and liquid fuels may not (Knothe, 2001). In 1925, Henry Ford wrote to The New York Times that in the future, fuel would come from things like weeds and sawdust and that fuel existed in all vegetable matter that can be fermented ("Ford Predicts Fuel from Vegetation", 1925). During World War II, when petroleum fuel supplies were interrupted, vegetable oil was used by several countries. Sweden developed cars that ran using wood gasification in the 1920s. Many vehicle companies experimented with this technology during World War II (Sikarwar et al., 2017). However, when the war ended, bioenergy fuels were replaced by petroleum. The petroleum industry offered cheap and plentiful fuel, which hindered progress of bioenergy development. Geopolitical conflicts cause renewed interest in biofuels when our reliance on fossil fuels becomes uncertain (Sikarwar et al., 2017).

Still, there are important considerations regarding energy from biomass. Investigating biomass crops for energy production has critical features: sustainability, high photosynthetic rates, potential for genetic improvement, efficient industrial conversion, and environmental friendliness are a few requirements. Many plant species are being investigated, but few have agronomic practices already developed, or they require genetic improvement to optimize biomass production. Additionally, some of these plants may require development of new technology for cultivation adding expense and time to the process. However, the use of existing domesticated crops does not require the same learning curve. Several food crops have already been investigated and used extensively as bioenergy feedstocks; among them are corn (*Zea*

mays) and sugarcane (Khan et al., 2016). Using food crops for bioenergy raises concerns about increasing the cost of food (Tenenbaum, 2008). This has directed research toward perennial, non-food bioenergy crops. Species in the *Saccharum* complex contain lignocellulosic biomass and produce high amounts of tonnage making them prime candidates for bioenergy crops.

Sugarcane

Physiology and Production

Sugarcane is grown in about 100 countries worldwide. It is the largest crop commodity regarding total production, occupying about 26.9 M ha. In controlled conditions, sugarcane can yield as much as 200 Mg ha^{-1} gross cane weight (Khan et al., 2016). In 2009, 1,682 M Mg were produced world-wide. Native to southcentral and southeastern Asia, sugarcane and has been cultivated in India for more than 5,000 years. It is a C_4 photosynthesizer, allowing for vigorous growth under high temperatures and humidity. It was introduced in the New World in the 16^{th} Century. In tropical latitudes, sugarcane takes 12-18 months to mature (Scortecci et al., 2012). It produces many stalks from each stool (tillers of a crown) and regrows after harvest (ratooning). The number and vigor of ratoon crops depend on cultivar, management, and environment (Olaoye, 2001). In Louisiana, two to four ratoon crops are economically possible (ASCL, 2020). Its plant architecture (height, erect growth habit, and ability to tiller) are important indicators of its value as a biomass crop (de Souza et al., 2013). Sugarcane produces glucose, which is converted and stored as sucrose, as much as 23% (w/v), in the vacuoles of parenchyma tissue in the stems. Sugarcane is considered a first-generation biofuel crop (fermenting sugars to ethanol). In addition to producing sugar, crushed stems and leaves are burned for electricity and steam to power sugarcane mills (Scortecci et al., 2012). However, liquid fuel production from this lignocellulosic residue, or bagasse, is theoretically possible. Cell walls are composed primarily

of cellulose. A study in Louisiana found the composition of energycane bagasse to be 43% cellulose, 24% hemicellulose, and 22% lignin (Kim & Day, 2011). Second-generation biofuels are produced from the breakdown of cellulose and hemicellulose into fermentable monomers of glucose and xylose (Scortecci et al., 2012). Starch is a chain of glucose molecules linked in α-(1,4) glycosidic bonds (Fig. 2.1; Taiz et al., 2015a). Cellulose is a tightly bound, three-dimensional arrangement of glucose molecules, but monomers are linked in β-(1,4) glycosidic bonds. Hydrogen bonds hold the cellulose chains in a highly organized, crystalline structure (Taiz et al., 2015b).

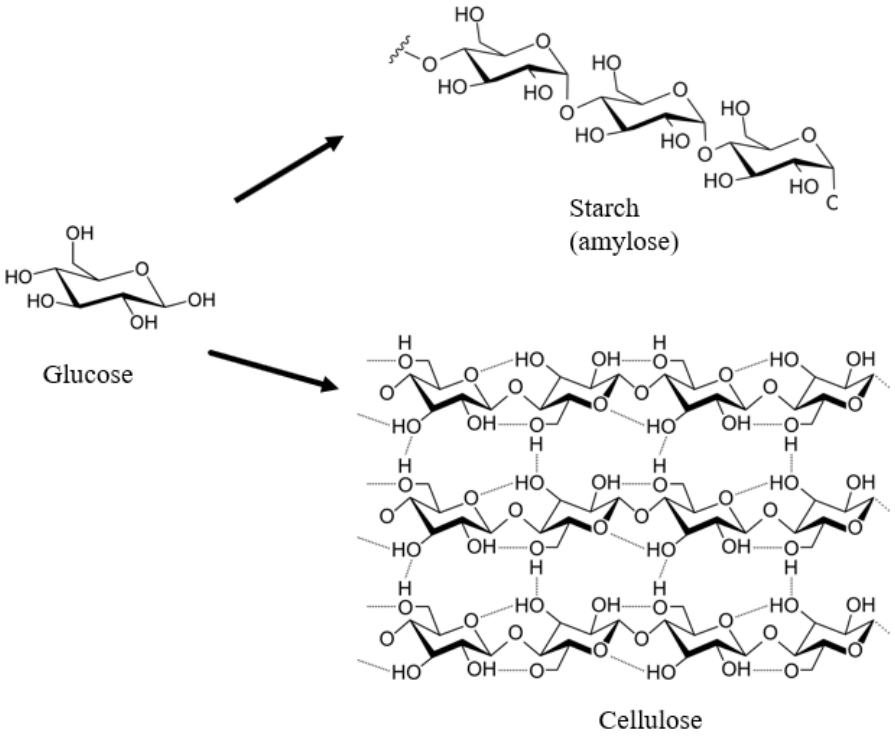

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Figure 2.1 Chair conformations of glucose, starch, and cellulose showing the relationship due to polymerization of glucose.

Breeding

Sugarcane breeding plays an essential role in both sugar production and ethanol production, from first- or second-generation material, by providing genotypes with superior traits. The release of a new cultivar may take 13 years (Scortecci et al., 2012). Sugarcane varieties have been bred largely for greater sucrose content and tonnage. However, desire for second-generation biofuels has enhanced development of varieties greater in fiber content, diversifying breeding programs to produce genotypes suited for marginal environments and colder climates. Another important goal for breeding is tolerance to abiotic and biotic stresses. Stress includes any situation that hinders expression of full genetic potential. Drought stress is a major concern for sugarcane. Although sugarcane can survive long periods of drought, regular rainfall is necessary for optimal production. Sixty percent of sugarcane fields in Australia and 40% in South Africa are irrigated. A study conducted by Texas A&M University showed irrigation of sugarcane greatly affected yield. Cane yield and sugar yield both increased as irrigation level increased (Wiedenfeld, 1995). If sugarcane cultivation is to increase, more drought tolerant genotypes will be needed for less favorable climates or inadequate access to water (Scortecci et al., 2012). In Louisiana, flood-tolerance is of greater concern than drought tolerance. Studies have shown that energycane is more tolerant to periodic flooding than sugarcane (Viator et al., 2012). Commercial sugarcane has little to no cold tolerance. There is limited growth under 10° C, and is damaged by freezing temperatures, therefore, breeding for cold tolerance involves two traits: winter survivability and growth at lower temperatures (Yates, 2015). Late spring emergence is another desirable trait for more northern latitudes to avoid early shoots from succumbing to late spring frosts (Baldwin, personal communication). Biotic stresses are of equal concern. As sugarcane varieties are improved, pests and pathogens change to

overcome these improvements. Biotic stresses have economic impact on production in the countries that depend on sugarcane as a source of fuel or revenue (Scortecci et al., 2012).

Inter-species hybridization is the main strategy to develop new genotypes for sugar, ethanol, or biomass production. The "*Saccharum* Complex" is an interbreeding group of species and closely related genera such as *Miscanthus*, *Erianthus*, and *Narenga*. One important species in the *Saccharum* Complex is *S. spontaneum* (Scortecci et al., 2012). This species' Center of Origin is India; however, it has a very wide distribution of genetically related species that range from the Mediterranean to Japan. *Saccharum spontaneum* is adaptable to many environments and shows resistance to several diseases. *Saccharum spontaneum* has a more rhizomatous habit and produces more tillers than commercial sugarcanes, making it important for biomass production. Its greater fiber content and more vigorous root system provide some genotypes with more tolerance to cold and water stress than commercial sugarcane. It is potentially a good candidate for genetic improvement of *Saccharum* for both sugar and fiber production; however, *S. spontaneum* is federally classified as a noxious weed and cannot be phenotyped in the field without first being introgressed into a domestic species (Hale, Personal Communication). The first generation (F_1) hybrids produce the greatest fresh and dry matter yields, and because they are vegetatively propagated, the hybrid vigor derived from the inter-specific crossing can be exploited (da Silva, 2017). *Saccharum spontaneum* has less sugar content than commercial sugarcane cultivars and therefore is not commercially viable for food production (da Silva, 2017). In Louisiana, the commercial variety CP 65-357 had mean °Brix values of 20.6 two years in a row, while energycane varieties L 79-1002 and L 79-1003 did not exceed a value of 16 in the same study (Giamalva et al., 1984). Its ease of cultivation and flexibility towards unfavorable

conditions are a trade-off, allowing energycane to grow in areas where commercial sugarcane would not be economically viable.

A high level of genetic diversity is another important aspect of *Saccharum* hybridization. A study conducted at the Fujian Agriculture and Forestry University revealed octoploidy in *S. officinarum* 'LA Purple' ($2n = 8x = 80$) and *S. spontaneum* 'SES208' ($2n = 8x = 64$) and demonstrated chromosomal rearrangements between the two species (Meng et al., 2019). Studies performed on the *Saccharum* Complex suggested that there are three basic genomes in *Saccharum* species: x= 6, 8, and 10. *Saccharum spontaneum* genomes have been reported from 2n = 48 to 112 (da Silva, 2017). *Saccharum officinarum* studies indicate chromosome numbers from 2n = 100 to 130 (Scortecci et al, 2012). Modern sugarcane varieties have also been shown to contain chromosomes from *S. spontaneum*. Up to 27.5% of the genome of sugarcane was shown to be derived from *S. spontaneum* with up to 13% of the chromosomes the result of inter-specific chromosome recombination (Piperidis et al., 2010). This variety of genome numbers gives an idea of the complex nature of sugarcane breeding.

Hybridizing

Hybridizing sugarcane with *S. spontaneum* began in the 1890's. The hybrid crosses between *S. sp.* and *S. spontaneum* that are developed for the purpose of biomass are referred to as "energycane. The first American released variety of energycane was by Louisiana State University, called L79-1002. The *S. spontaneum* parent came from the Himalayas (Bischoff et al., 2008). A breeding program (USDA-ARS) in Houma, LA has been using *S. spontaneum* to broaden the genetic base of sugarcane and produce improved cultivars since 1972 (da Silva, 2017). Data collected from a study in Puerto Rico indicated a total biomass increase of up to 100% and an increase in dry matter per hectare of 20% in inter-specific hybrids when compared

to the best yielding commercial sugarcane varieties (Carvalho-Netto et al., 2014). The extensive root system and prolific rhizome production of energycane genotypes, inherited from the *S. spontaneum* parent, gives the resulting hybrid better ability to exploit soil nutrients and provides a long-term sequestration of atmospheric carbon. Energycane can also inherit cold tolerance from its *S. spontaneum* parent. The species is found naturally in regions of Japan that normally experience sub-freezing weather from -6 to -11℃. Incorporation of *S. spontaneum* has the potential to increase ratooning ability and cold tolerance thus expanding the production range into cooler climates. Drought tolerance is another benefit inherited from *S. spontaneum* breeding. The species is also well adapted to semiarid Mediterranean landscapes, which may allow energycane biomass crops to grow in areas with little or no irrigation (da Silva, 2017). A study in Catania, Italy of *S. spontaneum* achieved yields of 9.6 Mg ha^{-1} and 17.9 Mg ha^{-1} gross cane weight in the first and second years, respectively; cellulose composed almost 37% of the structural polysaccharides (Scordia et al., 2014). Breeding programs (for sugar or biomass production) need to answer questions about the morphometrics and phenology, such as stand counts and heights, spring emergence, seasonal growth, and stalk composition (sugar and fiber content) of new genotypes quickly (Scortecci et al., 2012).

Biomass Industry and Technology

Cellulose is a polysaccharide consisting of β-(1,4) linked glucose units (Fig. 2.1). It represents about 10% of commercial sugarcane's fresh weight and about one third of its dry weight (Khan et al., 2016; Festucci-Buselli et al., 2007). Energycane hybrids between the sugarcane variety CP 52-68 (a Florida sugarcane) and the *S. spontaneum* variety Tainan 96 produced yields of more than 220 Mg ha^{-1} $year^{-1}$ with fiber content greater than twice that of commercial sugarcane parent (Giamalva et al., 1984). Both stalks and leaves can be used for

producing ethanol (fermented and cellulosic). Such an increase in cellulose could double net ethanol production when compared to traditional sugarcane varieties. The first step of second-generation ethanol is to decompose the cell wall into fermentable sugars. The industrial-scale version of this process is not well developed. The bagasse must undergo pretreatments in order for the cellulases to decompose the substrate (Khan et al., 2016). A study conducted at the Energy Bioscience Institute, University of Illinois, deemed it necessary to thermochemically pretreat lignocellulosic material prior to enzymatic hydrolysis to enhance efficacy and economy. The intense energy requirement of size reduction and pretreatment processes on lignocellulosic material raise concerns about the carbon-neutral benefits of energycane production (Vidal et al., 2011). Cellulases are produced by many microorganisms, especially bacteria and fungi. Research in cellulosic biomass has several aims: to develop genotypes with improved cell wall constitution and more cellulose; to improve methods for mechanical harvesting with less impact on the environment (agricultural sustainability); to improve bacteria and yeast strains with greater capacity for fermentation; to use the entire aerial plant; and to use pretreatments on residues for fermentation without exceeding the energy output of the product (Scortecci et al., 2012).

In the process of fermentation, yeast is recovered from a previous fermentation, and added to fresh juice. The mixture ferments for a few hours while ethanol is produced (Diaz et al., 2015). The yeast strain's tolerance to ethanol and other metabolic toxins limits the process (Nguyen et al., 2017).

Many countries have implemented programs to research and increase bioenergy production. Brazil began implementing sugarcane as a source of biofuel in 1933 with the creation of the Instituto do Açúcar e do Alcool (IAA), and re-implementation the program again in 1975 under the Proálcool program after the Oil Crisis of 1973. The government mandated

Petrobas, a large oil company, to blend ethanol with gasoline at a minimum of 22%. It provided low-interest loans and subsidies to stimulate ethanol production and reduce the cost to consumers. This pushed gasoline-run, light vehicles out of production and use (Hofstrand, 2009; Uchoa, 2014). The Proálcool program experienced several shifts in success until 2003 when the flex-fuel technology launched (de Souza et al., 2013). Flex-fuel technology allows cars to run on 100% gasoline, 100% ethanol or any mixture of the two. The sale of cars with flex-fuel technology increased to about 86% of all new car sales in Brazil (Matsuoka et al., 2009). Gasoline, as of 2007, is sold mixed at 25% ethanol throughout the country (Hofstrand, 2009). Ethanol accounts for 15% of Brazil's total liquid fuel consumption and 50% of light vehicle fuel consumption (Fedenko et al., 2013).

In the United States, the mandated goal is 136 B L of alternative liquid transport fuels by 2022. Corn ethanol is limited to 42% of that goal (57 B L yr^{-1}) (Fedenko et al., 2013). The United States have an already existing sugarcane industry, so technology for planting and harvesting is readily available. However, current price supports require sugarcane to be processed to refined, white granular sugar. Producing ethanol from granular sugar is not economically feasible (USDA, 2020).

Contemporary Concerns

Two major concerns with planting sugarcane as a bioenergy crop are land use and greenhouse gas emissions. Corn produces around 8.65 Mg ha^{-1} of starch, which yields around 3,800 L ha^{-1} of ethanol. Sugarcane, however, can produce up to 80 Mg ha^{-1} of sucrose or 7,000 L ha^{-1} of ethanol (de Souza et al., 2013). As a perennial C_4 crop, sugarcane produces the largest output:input ratio in bioenergy production (Khan et al., 2016). While energycane does not produce as much sucrose as commercial sugarcane varieties, it offers a greater contribution as a

second-generation ethanol option. Using cellulose from crushed stems and leaves as a second-generation ethanol source could double the contribution of sugarcane to the bioethanol industry (Matsuoka et al., 2010). Energycane has even greater potential. A study in Louisiana estimated ethanol production for sugarcane and energycane at 2,825 kg ha^{-1} and 10,130 kg ha^{-1}, respectively, due to the energycane's greater fiber production (Kim & Day, 2011). The net carbon balance of *Saccharum* species is also an important consideration. The national average output:input ratio for corn is 2:1 (Luo, 2016). The output:input ratio of sugarcane ethanol ranges from 8.2:1 to 10:1. Bolstering its carbon reducing potential is its ability to ratoon (Lee et al., 2018). In Mississippi, a study showed that energycane varieties decreased in mean dry matter yield after three ratoons (four production years), but in other locations, productive ratoons are possible for many more years (Lee et al., 2018; Olaoye, 2001). *Saccharum* also has greater carbon sequestration with increasing atmospheric concentration. Brazilian sugarcane varieties, when grown for 50 weeks in a controlled environment at double the [CO_2] increased photosynthesis by 30% and acquired 40% more biomass. This alleviates concerns about greenhouse gas emissions (Scortecci et al., 2012).

Land use is an economic and social issue. In Brazil, sugarcane occupies 2% of agricultural land. To meet the country's internal and global demands for energy, at least double that amount was needed. Use of marginal land is a requirement for the expansion of bioenergy crops to prevent the displacement of food production. Lower input, perennial biomass crops are also a necessity (Scortecci et al., 2012). Since its introduction to Brazil in 1532, cultivation of sugarcane on low fertility lands provided natural selection for *Saccharum* genotypes that form associations with nitrogen-fixing bacteria, and thus, have little need for nitrogen inputs (Baldani et al., 2002). Several bacterial genera have been found to associate with sugarcane including

Gluconacetobacter diazotrophicus. This bacteria colonizes external root tissue and invades the transpiration stream via the xylem. These bacteria have been shown to significantly increase nutrient uptake and provide significant amounts of nitrogen to the plant. A study in India showed that inoculation increased germination of seed canes, tiller number, and mean height of a commercial sugarcane variety CoSe92423, and N tissue concentration was 7.32 to 21.97% greater than the control (Suman et al., 2005). Under low fertility situations, inoculation of *Saccharum* genotypes with associative nitrogen-fixing bacteria may decrease need, and therefore, cost of nitrogen fertilizer application (Eskin et al., 2014; James et al., 1994; Suman et al., 2005).

Methods of Biomass Conversion

Another area of research concerning the production of biofuel from energycane is methods of analysis and decomposition of cellulose. There are several available methods to catabolize cellulose in bagasse and quantify the products of decomposition. Products depend on the feedstocks used and the parameters of the catabolic process (He et al., 2019; Ball et al., 1991). After extractable sugars and cellulose have been converted, the lignin material leftover can be burned for energy (Antizar-Ladislao & Turrion-Gomez, 2008).

Pyrolysis

Pyrolysis is thermal decomposition of organic matter in the absence of oxygen (Arni, 2018). The products of pyrolysis are gas, liquid, and biochar. Pyrolysis can be divided into four categories: slow, intermediate, fast, and flash pyrolysis. Slow pyrolysis reaction times may be hours to days, heating at a rate of less than 1° C s^{-1}. Intermediate pyrolysis occurs in minutes at a heating rate of 5 to 50° C s^{-1}. Fast pyrolysis occurs in seconds at more than 200° C s^{-1}. Flash

pyrolysis occurs in less than two seconds at more than 500° C s^{-1} (He et al., 2019). Slow pyrolysis favors solid biochar production. Biochar can be applied to soil as a conditioner. It can increase soil fertility and water-holding capacity. The process of producing biochar from agricultural residue or biomass is sustainable and can mitigate greenhouse gas emissions (He et al., 2019). Fast pyrolysis favors bio-oil production. Depending on the composition of the feedstock, 60-95% of the wet weight is converted into bio-oil. Greater lignin content results in lower bio-oil production. (Arni, 2018). Gasification is third thermal decomposition which involves fast pyrolysis. Gasification includes controlled addition of oxygen resulting in production of syngas exclusively (He et al., 2019).

Simultaneous Saccharification and Fermentation

Simultaneous saccharification and fermentation is the practice of digesting cellulose with cellulase enzymes in the presence of microorganisms that can ferment sugars as well. The process saves time and money by consolidating the two processes and removing end-product inhibition of the saccharification process (alcohols and esters). The process has shorter production time, greater ethanol yield, and less energy consumption than the processes run sequentially. This process, however, is inhibited by toxic by-products that reduce the efficiency of microbial fermentation (Brown et al., 1981; Haq et al., 2016; Visioli et al., 2014).

Hydrolysis

Hydrolysis is a feedstock pretreatment used to improve saccharification and increase hydrolysis of cellulose by breaking down lignin and hemicelluloses, and thus increasing the porosity of the cell wall (dos Santos et al., 2019; Sun & Cheng, 2002). Two important kinds of hydrolysis are acidic and enzymatic. Acid hydrolysis is performed in two main ways:

concentrated and dilute. Acid hydrolysis uses strong mineral acids like HCl and H_2SO_4. It can be performed at high concentrations coupled with low temperatures or low concentrations at high temperatures. Concentrated acid hydrolysis is expensive, requiring corrosion-resistant equipment, neutralization, and acid recovery procedures to reduce cost. Dilute acid hydrolysis is more cost efficient but produces fermentation-inhibiting compounds that can reduce yield by poisoning fermentation organisms. Concentrations, temperatures, type, and composition of substrate all affect the amount of free sugars produced (Haq et al., 2016).

Methods of Biomass Analysis

Van Soest Fiber Analysis

Digestibility gives information on the composition of the cell wall structure and its value as a bioenergy feedstock (He et al., 2019; Ball et al., 1991). Van Soest is a kind of proximate analysis in which the digestible components of plant material are separated from the indigestible components. It uses two detergents. First, a neutral detergent to separate cell contents (protoplast) from cell wall. These components together are known as neutral detergent fibers (NDF). An acid detergent is used next to separate hemicelluloses (acid detergent fiber or ADF) from other cell wall components (cellulose and lignin) (Holechek & Vavra, 1982; Goering & Van Soest, 1970). Sulfuric acid is used after to dissolve cellulose, leaving behind the acid detergent lignin (ADL). Percentages of cellulose, hemicellulose, and lignin are calculated by subtraction (Hatfield et al., 1994). The sample is dried and weighed between each process in order to determine the percentage of each constituent (Holechek & Vavra, 1982; Goering, & Van Soest, 1970). Crude protein is measured by heating the sample to 420 °C and adding sulfuric acid, then sodium hydroxide to neutralize the acid and liberate ammonia (Kjeldahl). The ammonia is distilled and titrated to measure its concentration (Thiex et al., 2002).

Near Infrared Spectroscopy

Near infrared spectroscopy (NIRS) is an instrumental technique used to determine nutrient makeup of plant tissues. Hydrogen bonds absorb energy at certain wavelengths that cause the bonds to become excited and vibrate. Wavelengths used are between 1200-2500 nm (Norris, 1989). Upon this excitement the bonds emit energy back at a certain wavelength. Near infrared spectroscopy shines near infrared light onto a sample of plant material. The NIR light that is given off from the sample is received and fit to known curves to determine the chemical makeup of the sample (Barton, 1989; Ball et al., 2015). It can be used to estimate acid detergent fibers, neutral detergent fibers, fats, crude protein, sugars, and lignin (Ball et al., 2015). Its accuracy relies on previous databases and calibrations for specific materials based on wet chemistry of van Soest (Barton, 1989).

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CHAPTER III

MATERIALS AND METHODS

Planting Material and Study Site

This research was conducted at Mississippi State University RR Foil Plant Science Research Center (2018) (33.467952, -88.754920), the HH Leveck Animal Research Center (2019) (33.423582, -88.792412) and the Bearden Dairy Research Center (2020) (33.39499, -88.74101) near Starkville, MS. The germplasm in this study was derived from energycane breeding material that was provided by Dr. Anna Hale (USDA-ARS, SRU; Houma, LA). This germplasm was selected as seedlings for °Brix and visual performance at the USDA-ARS Ardoyne Research Farm near Schriever, LA and planted into first-clonal trials. The following year, 20 individuals from 50 families (1000 genotypes) were collected from the plant cane first-clonal stage and transported to the LSU Macon Ridge Research Station (32.1422701, -91.7087844; Winnsboro, LA) for evaluation and selection under freezing conditions. Selections were planted into 1.8m unreplicated plots for evaluation in the plant cane year. Selections were made based on stalk population, vigor, and disease resistance. Selections were planted into a second-clonal stage unreplicated trial in 4.9 m plots for evaluation in the plant cane through second-ratoon crop. During this time, winter temperatures fell to -7.3 °C. Genotypes were selected based on agronomic type, vigor, and stalk population. In addition, 10-stalk samples were collected and transported to the lab at the Ardoyne Research Farm in Houma, Louisiana to determine fiber content, °Brix, sucrose content, stalk weight, and moisture. These measurements

were used to calculate total fresh weight ha^{-1}, total dry matter ha^{-1}, and theoretical ethanol ha^{-1}. Twenty-six genotypes were selected from this material during the second ratoon. They were assigned the designations of AFRI15-1 through AFRI15-26. Two varieties served as controls: Ho02-113 as the energycane control and L01-299 as the sugarcane control. Canes of the twenty-six genotypes and controls (Table 1) were planted at RR Foil Plant Science Research Center in September 2018. The genotypes were planted as whole-stalks which were topped below the apical meristems in rows 6.1 m long and 1.8 m on center. All subsequent seed cane came from these plots. The soil is a Leeper silty clay loam classified as fine, smectitic, nonacid, thermic Vertic Epiaquepts.

Table 3.1 The 28 genotypes planted in the fall of 2018 to assess survivability, yield, and theoretical ethanol production.

Original 28 Genotypes			
AFRI15-1	AFRI15-8	AFRI15-15	AFRI15-22
AFRI15-2*	AFRI15-9	AFRI15-16*	AFRI15-23
AFRI15-3	AFRI15-10*	AFRI15-17*	AFRI15-24
AFRI15-4	AFRI15-11	AFRI15-18	AFRI15-25
AFRI15-5	AFRI15-12	AFRI15-19	AFRI15-26*
AFRI15-6	AFRI15-13	AFRI15-20	Ho02-113
AFRI15-7	AFRI15-14*	AFRI15-21	L01-299*

Asterisk indicates genotype that was not carried on to the replication trial at HH Leveck Animal Research Center or at Bearden Dairy Research Center.

Twenty of these genotypes and one control (Ho02-113) were selected for further testing in fall 2019. The other six genotypes and sugarcane control (L01-299) were not carried forward for further testing due to lodging or low yield. The 20 selected genotypes and controls were planted in a randomized complete block design with four replications at HH Leveck Animal Research Center in the fall of 2019. The soil at this location is a Catalpa silty clay loam, classified by fine, smectitic, thermic Fluvaquentic Hapludolls. Rows were 12.19 m (40 ft) long

and 1.83 m (6 ft) on center. To plant at this location, furrows and beds were pulled across the entire planting site. Beds, and therefore furrows, were spaced 0.92 m apart. Seed canes were harvested using a machete. The distal end of the cane was removed so that each planted cane measured either 1.83 m, 2.44 m, or 3.05 m depending on height of the canes. Canes that were 1.83 m, were planted 10 to a 12.19 m plot, 2.44 m planted seven to a plot, and 3.05 m planted six to a plot. Canes were laid in every other furrow overlapping by approximately one third of each cane length. Canes were covered with soil using a three-point tilt scraper blade. Soil cores were taken and analyzed for soil nutrients. Nitrogen (UAN 30-0-0) was applied at a rate of 168.13 kg ha^{-1} using a knife rig on 17 June 2020. The same genotypes were carried into a new replicated field test located at Bearden Dairy Research Center (fall of 2020). The same harvesting and replanting techniques were used at this location. Soil at Bearden Dairy is a Kipling silty clay loam classified as fine, smectitic, thermic Vertic Paleudalfs.

Data Collected

Stalk Heights and Counts

Stalk heights and stand densities were recorded biweekly starting on 12 June 2020 and ending on 16 October 2020. Mean heights were plotted to visualize relative growth, rate of growth, and determine the onset and cessation of the grand growth period. Heights were taken via random sample of five canes $plot^{-1}$ and the mean of each genotype was calculated. Heights were measured (cm) from the ground to the most distal set of auricles (the dewlap). Stand densities were obtained by counting each stalk found in each plot and extrapolating to stalks ha^{-1}.

Biomass Composition and Yield

End-of-season harvest was performed using a Cibus S Wintersteiger plot harvester (Ried im Innkreis, Austria) in 2020 and 2021. Each plot was harvested and weighed. A sub-sample of chopped cane from each genotype was obtained, weighed, and dried for moisture determination. A random, "millable" cane was sampled from each plot in each replication for height, weight (kg), and diameter of the lowest internode (mm). A "millable" cane was defined as a cane that had senesced leaves (not a recent tiller), and whose height was mean for the plot (dewlap reached the canopy). These sample stalks were crushed in a three-roller electric sugarcane juicer (Plant Based Pros®; Jersey City, NJ). The total sap volume was stirred in order homogenize the sample for a representative °Brix value. The °Brix value was recorded with a digital refractometer. Total extracted juice volume (mls) for each stalk was recorded. After juicing, crushed stalks were weighed, dried to completion, and weighed again. Data was extrapolated to units ha^{-1}. The data from the 2020 plant cane (PC) year at the HH Leveck Animal research Center were compared to 2021 PC year at the Bearden Dairy Research Center and the 2021 ratoon year at the HH Leveck Animal Research Center.

Theoretical Ethanol Yield

To predict first-generation (fermentable) theoretical ethanol yield (TEY) from carbohydrates in the sap, Equation 1 was used. Sap volume from the sample cane was multiplied by stalks ha^{-1} to get L ha^{-1}. This was multiplied by the mean °Brix reading as a percentage from the plot sample cane to obtain soluble sugars ha^{-1}. Soluble sugars ha^{-1} was multiplied by 0.75 assuming that 75% of the °Brix reading was fermentable sugar (Wortmann et al., 2010). This value was then multiplied by 0.581 according to the stoichiometry of yeast fermentation. The equation for first-generation TEY was:

$$\text{Sap volume (L ha}^{-1}\text{) x (°Brix x 0.75) x 0.581 = theoretical ethanol (L) ha}^{-1} \quad (3.1)$$

(Wortmann et al., 2010)

To predict second-generation TEY from cellulosic biomass (Equation 2), the dry matter (Mg ha^{-1}) was multiplied by 174.2 to get ethanol L ha^{-1}. The number 174.2 is a constant based on current hydrolytic second-generation technology (Dias et al., 2012) (Equation 2). The equation for second-generation TEY was:

$$\text{Mg ha}^{-1} \text{ x } 174.2 = \text{L ha}^{-1} \quad (3.2)$$

(Dias et al., 2012)

Total theoretical ethanol yield for both fermentation and second-generation simply requires the addition of the TEY from sap and the TEY from cellulose in dry matter. Data were analyzed using PROC MEANS and PROC GLM procedures for means separation at $\alpha = 0.05$.

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CHAPTER IV

RESULTS

Data Separated by Year and Location

There were three years of data assessed: the PC year at HH Leveck Animal Research Unit (harvested in 2020), the PC year at the Bearden Dairy Research Unit (harvested in 2021), and the ratoon year of the HH Leveck Animal Research Unit (harvested in 2021). Results were presented in this order. Data between PC locations were then compared to each other, as well as data between PC year and ratoon year for the first location (HH Leveck Animal Research Center). Analysis of variance indicated differences between PC locations and between years ($\alpha = 0.05$). Therefore, the data is organized by subject so that data across years can be compared.

Heights and Growth Curves

Heights measured at the HH Leveck Animal Research Center in 2020 cannot accurately display growth curves. Random height measurements included younger tillers throughout the growing season. Therefore, the heights displayed accurately represent the mean height of canes for a particular genotype across the growing season. However, this information is difficult to interpret because mean height (Fig. 4.1, 4.2, and 4.3) does not display the rate at which the canes grew. The random nature of cane selection and measuring allowed the incorporation of younger tillers, which decreased the mean height. Because of this, canes were flagged so that the same canes were measured throughout the growing season, and a rate of growth could be calculated. Height data is displayed by mean height taken across growing season by genotype, mean of all

genotypes, and growth rate across growing season. Data omitted from 27 Aug. to 4 Sep. 2020 in the PC year at the HH Leveck Animal Research Center (Fig. 4.1) occurred due to mandatory Covid-19 quarantine.

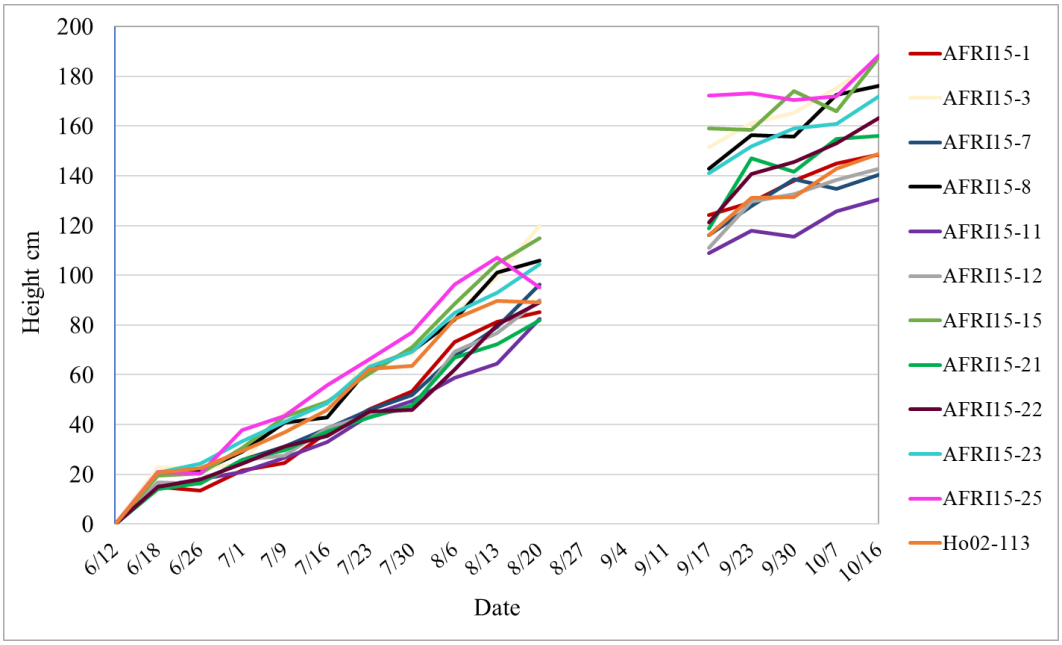

Figure 4.1 Mean height of various genotypes during growing season of the plant cane year at the HH Leveck Animal Research Center in 2020. The gap in data from 27 Aug to 11 Sept was due to mandatory Covid-19 quarantines.

Figure 4.2 demonstrates that cane growth among genotypes for the PC year at the Bearden Dairy Research Center (2021) was less variable than the PC year (2020) and first ratoon year (2021) at the HH Leveck Animal Research Center (Fig. 4.3). The same genotypes are demonstrated in Figures 4.1, 4.2, and 4.3. Mean rate of growth measured at the Bearden Dairy Research Center (2021; Fig. 4.4) and ratoon year of the HH Leveck Animal Research Center (2021; Fig. 4.5) more accurately demonstrate a growth curve and a period of "grand growth." Grand growth was defined as the period during which the rate of growth is increasing.

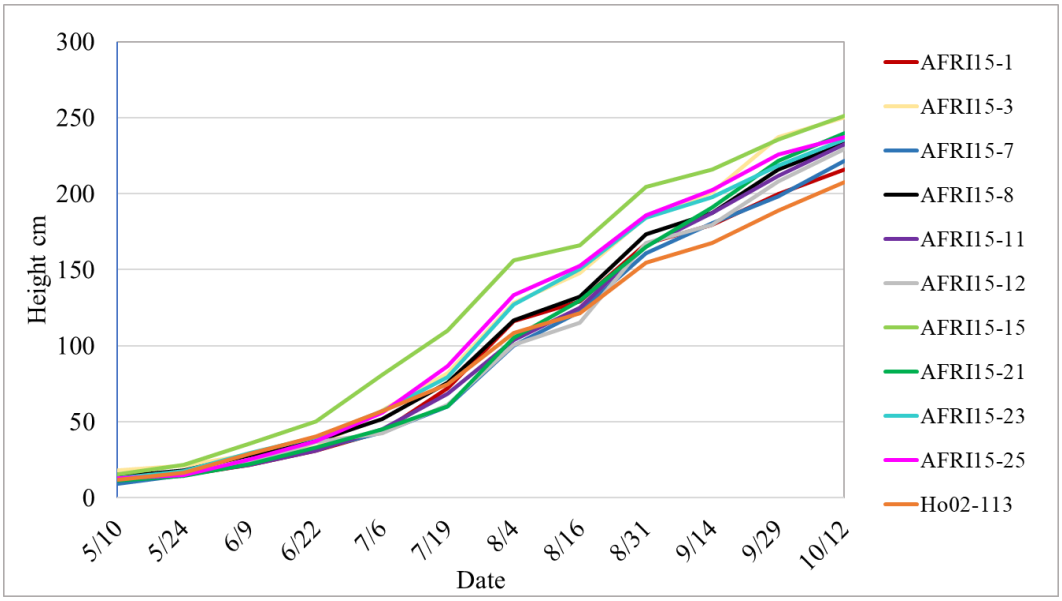

Figure 4.2 Mean height (cm) for 10 of 20 genotypes and control (Ho 02-113) during growing season in the plant cane year at the Bearden Dairy Research Center in 2021.

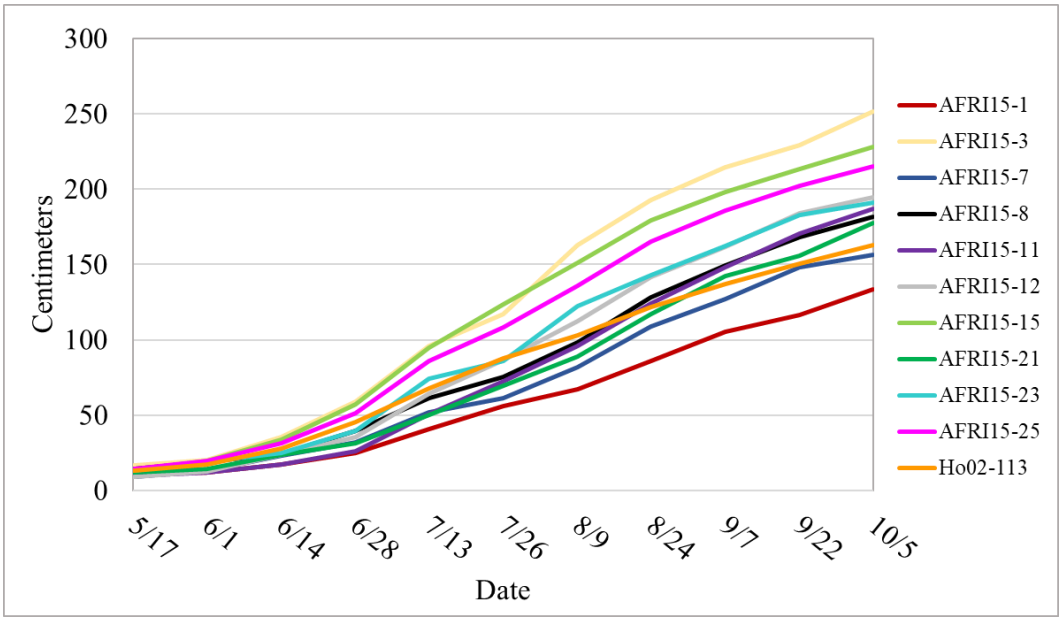

Figure 4.3 Mean height (cm) for 10 of 20 genotypes and control (Ho 02-113) during growing season in the ratoon year at the HH Leveck Animal Research Center in 2021.

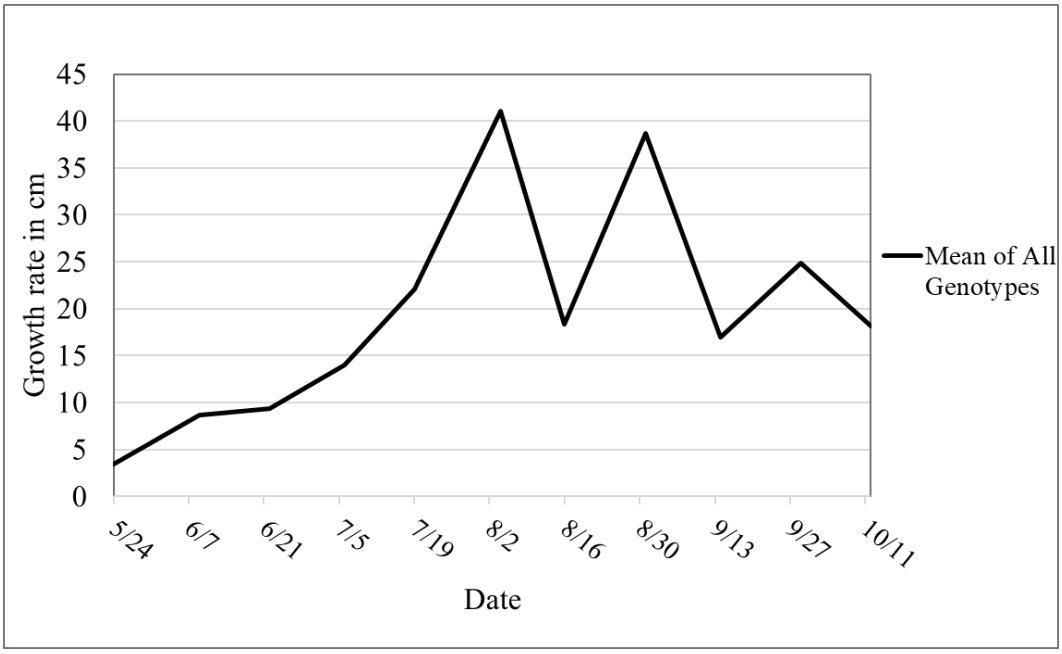

Figure 4.4 Mean rate of growth of all genotypes at the Bearden Dairy Research Center during the PC year (2021). Grand growth is demonstrated from 21 June 2021 to 2 August 2021.

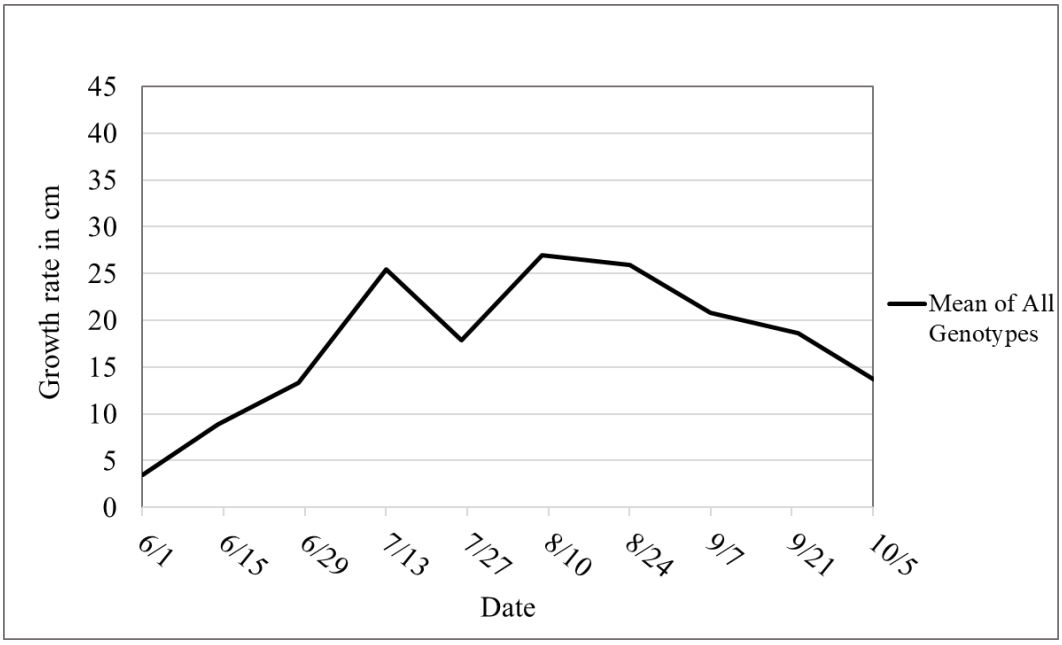

Figure 4.5 Mean rate of growth of all genotypes at the Bearden Dairy Research Center during the ratoon year (2021). Grand growth is demonstrated from 1 June 2021 to 10 August 2021.

Fresh Weights

There were significant differences in fresh weights (FW) among genotypes for the PC year at the HH Leveck Animal Research Unit ($P < 0.0001$; Fig. 4.6). The mean FW across all genotypes was 47.29 Mg ha^{-1}. The greatest mean yield was 61.05 (AFRI15-25), and the least was 19.94 Mg ha^{-1} (AFRI15-9). The FW of Ho02-133 was 51.56 Mg ha^{-1}. At this location, no genotypes produced significantly greater FW than the control; however, there were five genotypes (as indicated by the red asterisks on Fig 4.6) that produced significantly less FW than the control. There was an effect on mean regarding FW ($P < 0.0001$). Mean yields across genotypes increased from the first rep. (35.89 Mg ha^{-1}) to the fourth rep. (57.29 Mg ha^{-1}).

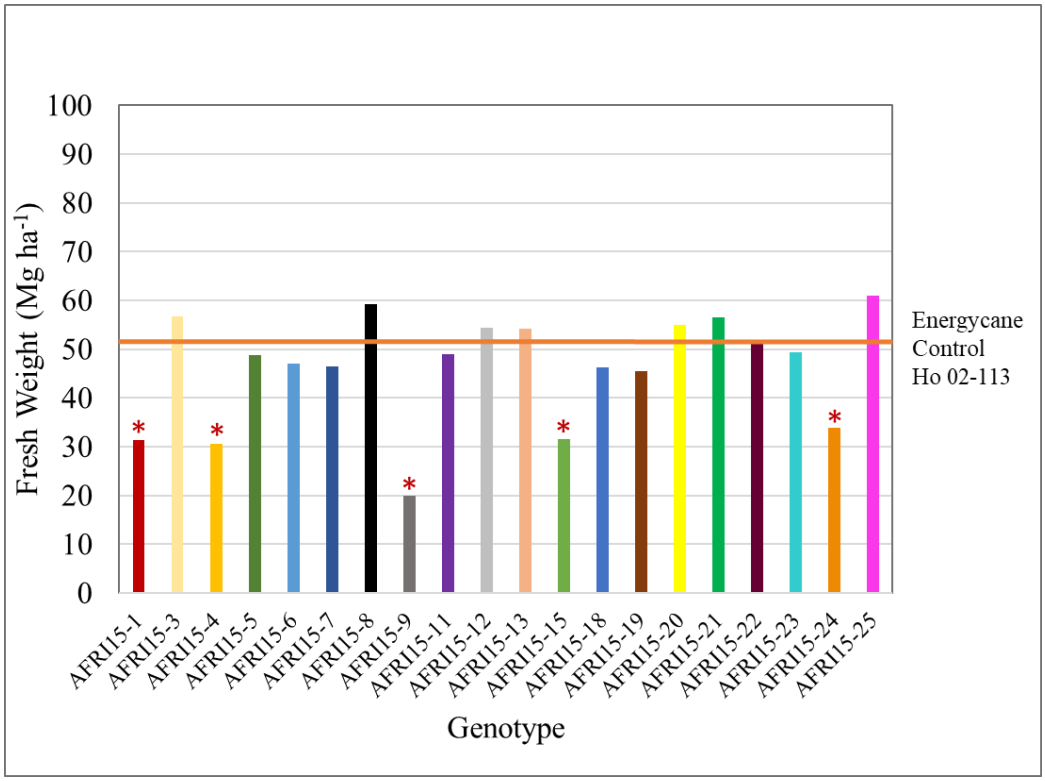

Figure 4.6 Mean fresh weight yields (Mg ha^{-1}) for the plant cane year at the HH Leveck Animal Research Center in 2020. Red asterisk indicates genotype that was significantly lesser in mean fresh weight yield than the control. $P < 0.0001$. LSD = 17.472.

There were significant differences in FW among genotypes for the PC year at the Bearden Dairy Research Unit in 2021 (Fig. 4.7; P = 0.0001). The mean FW across all genotypes was 67.1 Mg ha^{-1}. The greatest mean yield was 87.26 Mg ha^{-1} (AFRI15-7), and the least was 42.67 Mg ha^{-1} (AFRI15-23). The FW of the control Ho02-133 was 73.72 Mg ha^{-1}. None of the genotypes produced significantly greater FW than the control; however, there were four genotypes (as indicated by the red asterisks in Fig. 4.10) that produced significantly less FW than the control. Replications were significantly different regarding FW (P < 0.0415). The second replication had the greatest mean FW yield (71.45 Mg ha^{-1}), and the first replication had the least mean FW yield (61.12 Mg ha^{-1}).

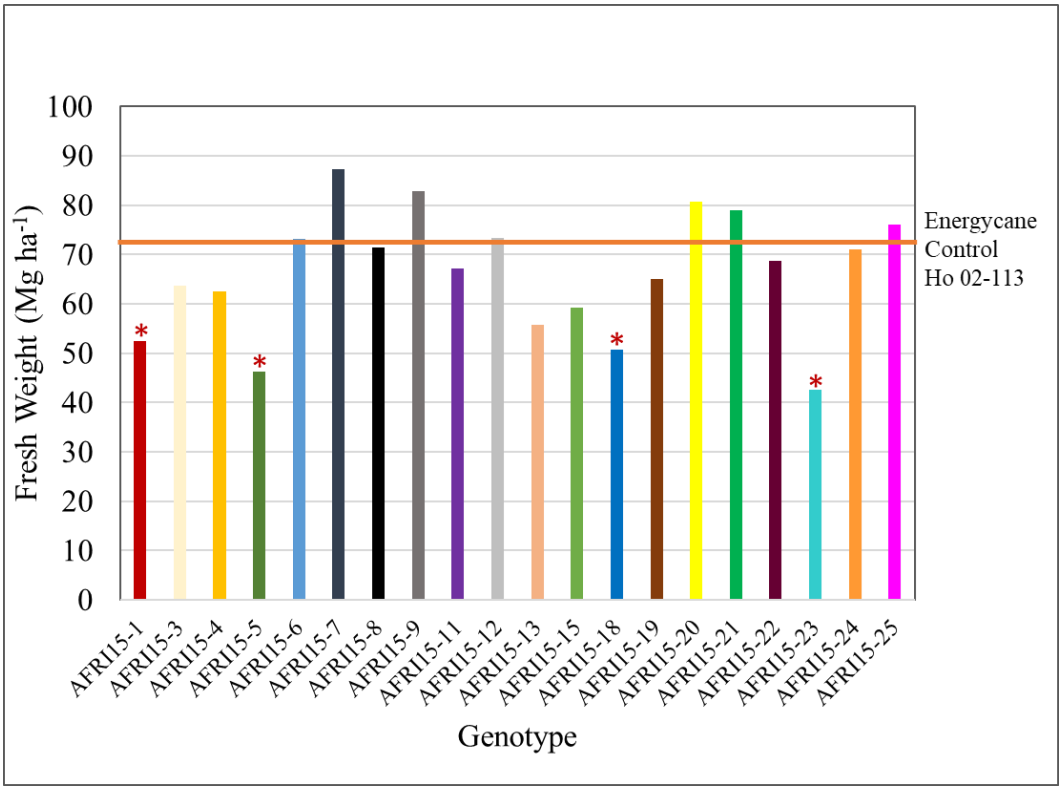

Figure 4.7 Mean fresh weight yields (Mg ha^{-1}) for plant cane year at Bearden Dairy Research Center in 2021. Red asterisk indicates genotype that was significantly lesser in mean fresh weight yield than the control. P = 0.0002. LSD = 18.51.

There were significant differences in FW among genotypes for the ratoon year at the HH Leveck Animal Research Unit in 2021 (Fig. 4.8; $P < 0.0001$). The mean FW across all genotypes was 34.28 Mg ha^{-1}. The greatest mean yield was 79.6 Mg ha^{-1} (AFRI15-3), and the least was 2.56 Mg ha^{-1} (AFRI15-1). The FW of the control Ho02-133 was 41.93 Mg ha^{-1}. AFRI15-3 yielded significantly greater than all other genotypes, and there were three genotypes (as indicated by the red asterisks in Fig. 4.11) that produced significantly lesser FW than the control. Replications were significantly different regarding FW ($P < 0.0205$). The second replications had the least mean FW yield (27.64 Mg ha^{-1}), and the third replication had the greatest mean FW yield (43.2 Mg ha^{-1}).

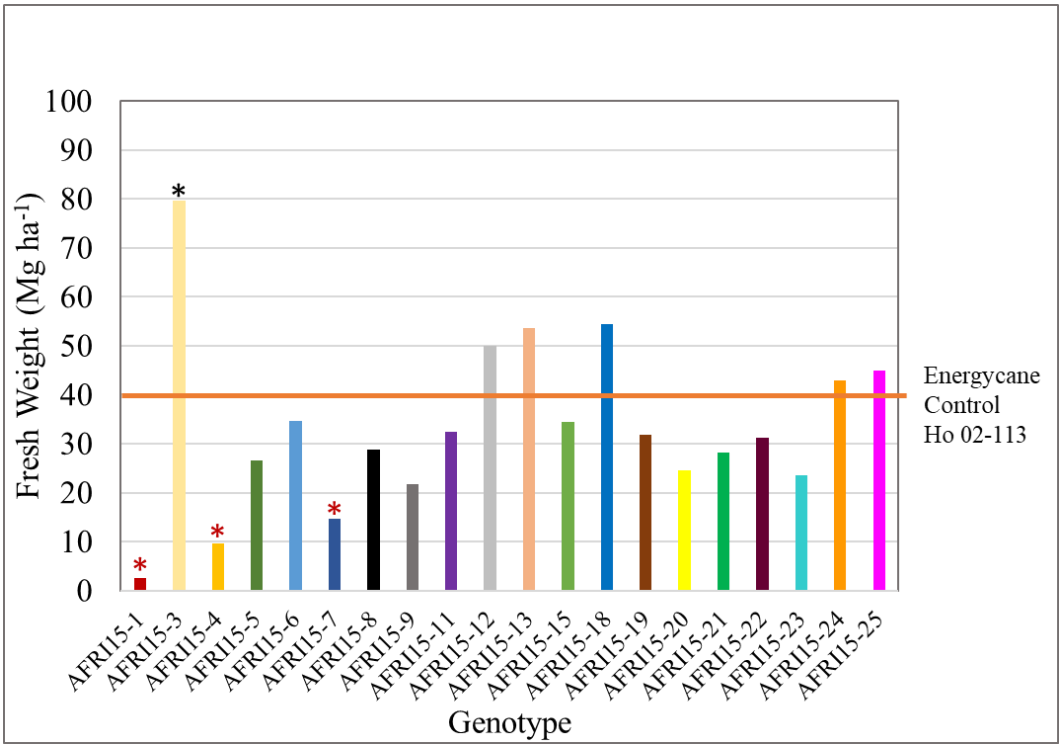

Figure 4.8 Mean fresh weight yields (Mg ha^{-1}) for first ratoon at HH Leveck Animal Research Center in 2021. AFRI15-3 yielded significantly greater fresh weights than other genotypes (black asterisk). Red asterisk indicates genotype with significantly lesser mean fresh weight yield than the control. $P < 0.0001$. LSD = 23.916.

Dry Matter Yield

There were significant differences in dry matter yield (DMY) among genotypes for the PC year at the HH Leveck Animal Research Unit ($P < 0.0001$; Fig. 4.9). The mean DMY across all genotypes was 11.104 Mg ha^{-1}. The greatest mean DMY was 15.175 Mg ha^{-1} (AFRI15-25). The least DMY was 3.975 Mg ha^{-1} (AFRI15-9). The DMY of the control Ho02-133 was 12.342 Mg ha^{-1}. No genotype produced significantly greater DMY than the control; however, there were four genotypes (as indicated by the red asterisks) that produced significantly less DMY than the control. There was also an effect on replication ($P < 0.0001$). Mean yields across genotypes increased from the first rep. (7.9 Mg ha^{-1}) to the fourth rep. (13.8 Mg ha^{-1}).

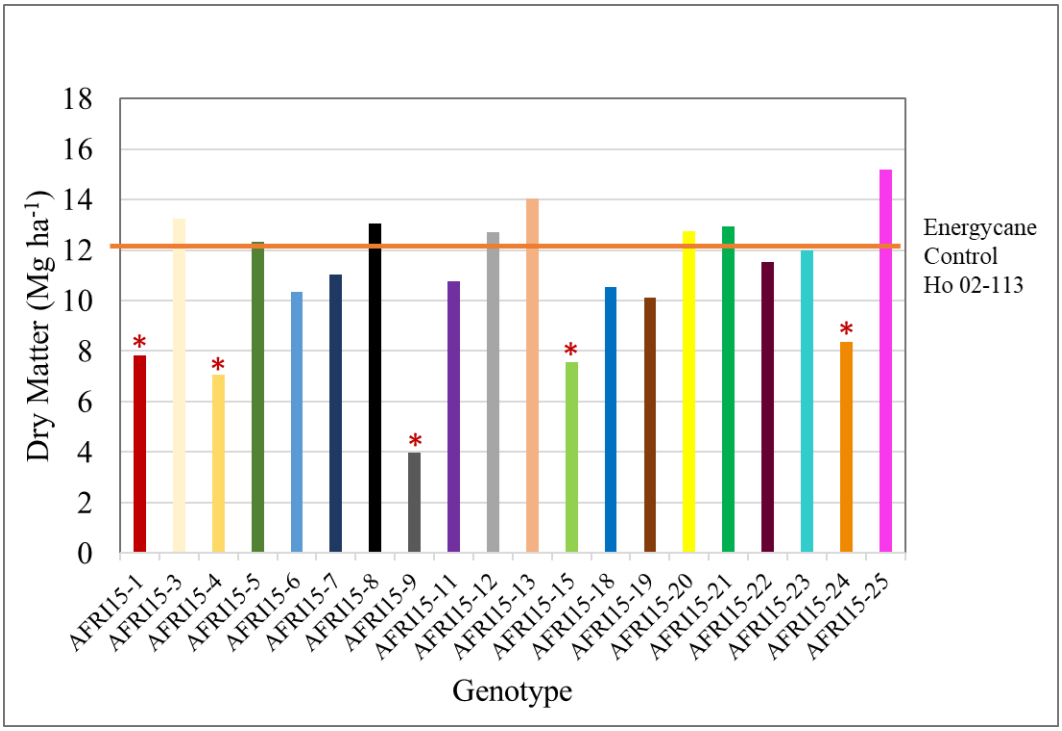

Figure 4.9 Mean dry matter yields (Mg ha^{-1}) for the plant cane year of the HH Leveck Animal Research Center in 2020. Red asterisk indicates genotype that was significantly lesser in mean dry matter yield than control (represented by orange horizontal line. $P < 0.0001$ LSD = 4.4669

Dry matter yields were significantly different among genotypes (P = 0.0010) in the PC year at the Bearden Dairy Research Center (2021; Fig. 4.10). The mean DMY was 13.71 Mg ha^{-1}. The mean DMY for the control was 16.06 Mg ha^{-1}. The greatest DMY was 16.948 Mg ha^{-1} (AFRI15-7). The least DMY was 8.597 Mg ha^{-1} (AFRI15-23). There were no genotypes that produced significantly greater DMY than the control, there were four genotypes (indicated by the red asterisks) that produces significantly lesser yields than the control. Mean DMY was significantly different among replications (P = 0.0117). Mean DMY by replication ranged from 12.1778 Mg ha^{-1} (first rep.) to 14.812 Mg ha^{-1} (second rep.).

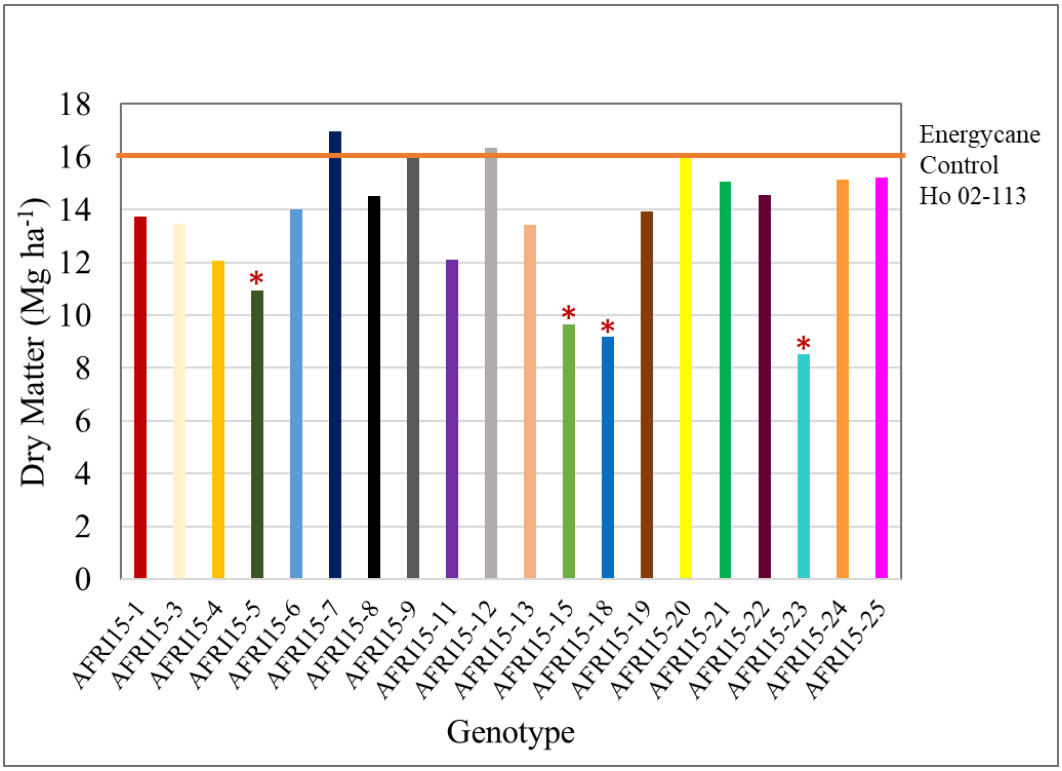

Figure 4.10 Mean dry matter yield (Mg ha^{-1}) for the plant cane year at the Bearden Dairy Research Center in 2021. Red asterisk indicates genotype that was significantly lesser in mean dry matter yields than the control (represented by the orange horizontal line. P = 0.0010 LSD = 4.1429

Dry matter yields were significantly different among genotypes ($P < 0.0001$) at the HH Leveck Animal Research Center in the first ratoon year (harvested in 2021; Fig. 4.11). The mean DMY was 9.399 Mg ha^{-1}. The mean DMY for the control was 12.425 Mg ha^{-1}. The greatest Mean DMY was Mg ha^{-1} (AFRI15-3). The least DMY was 0.364 Mg ha^{-1} (AFRI15-1). AFRI15-3 was the only genotype with significantly greater yield than the control, but there were four genotypes with significantly less DMY than the control (indicated by red asterisk in Fig. 4.11). There was also some effect due to replication ($P = 0.0225$). Mean DMY for the second replication was 7.574 Mg ha^{-1}. Mean DMY for the third replication was11.787 Mg ha^{-1}.

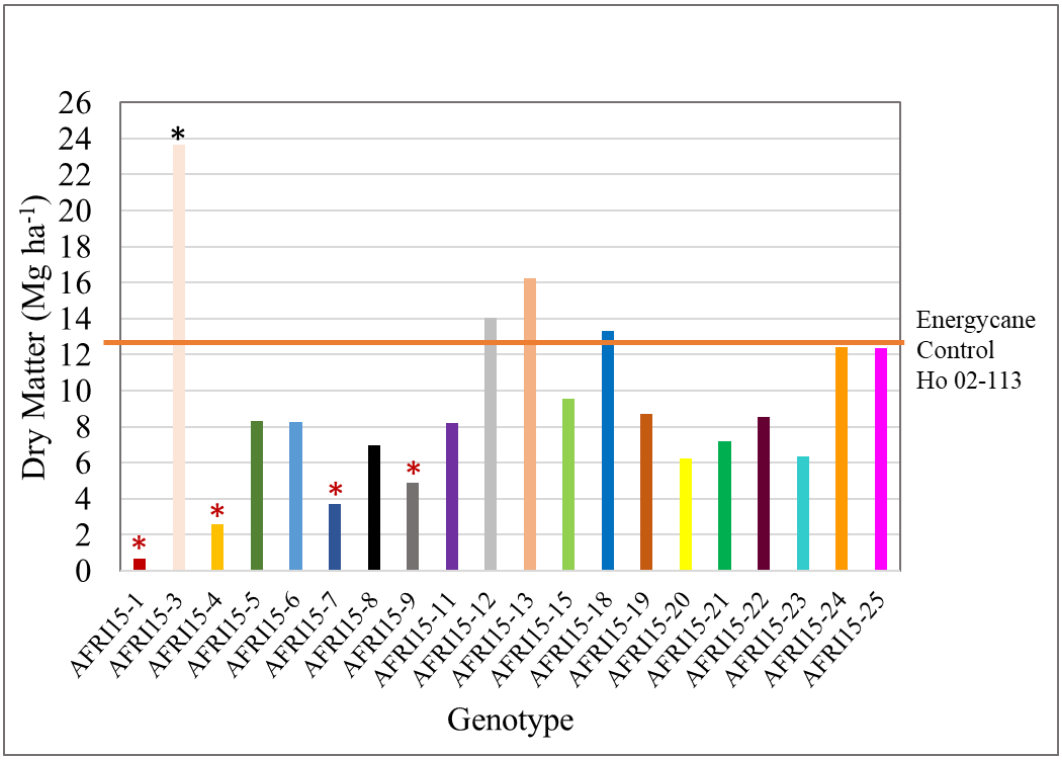

Figure 4.11 Mean dry matter yields in (Mg ha^{-1}) for the ratoon year at the HH Leveck Animal Research Center in 2021. AFRI15-3 yielded significantly greater than all other genotypes (indicated by black asterisk). Red asterisk indicates genotype that was significantly lesser in mean dry matter yield than the control (represented by the orange horizontal line). $P < 0.0001$. LSD = 6.679.

Sap Volume

Although energycane is not a sugar crop, sap volume is an important quality for future breeding strategies. Dissolved solids in the sap are the source of first-generation ethanol in sugarcane. There were significant differences in sap volume (SV) among genotypes for the PC year at HH Leveck Research Unit in 2020 ($P < 0.0001$; Fig. 4.12). The mean SV across genotypes was 0.31 ml g^{-1} of fresh cane. The greatest mean SV was 0.38 ml g^{-1} (AFRI15-8). The least mean SV was 0.23 ml g^{-1} (AFRI15-12). The mean SV for the control was 0.24 ml g^{-1}. There were 15 genotypes with significantly greater volumes of SV than the control. As expected, due to the replication effect on yield, there was also an effect due to replication on mean SV production. The mean SV increased from the first rep. (0.29 ml g^{-1}) to the fourth rep. (0.33 ml g^{-1}).

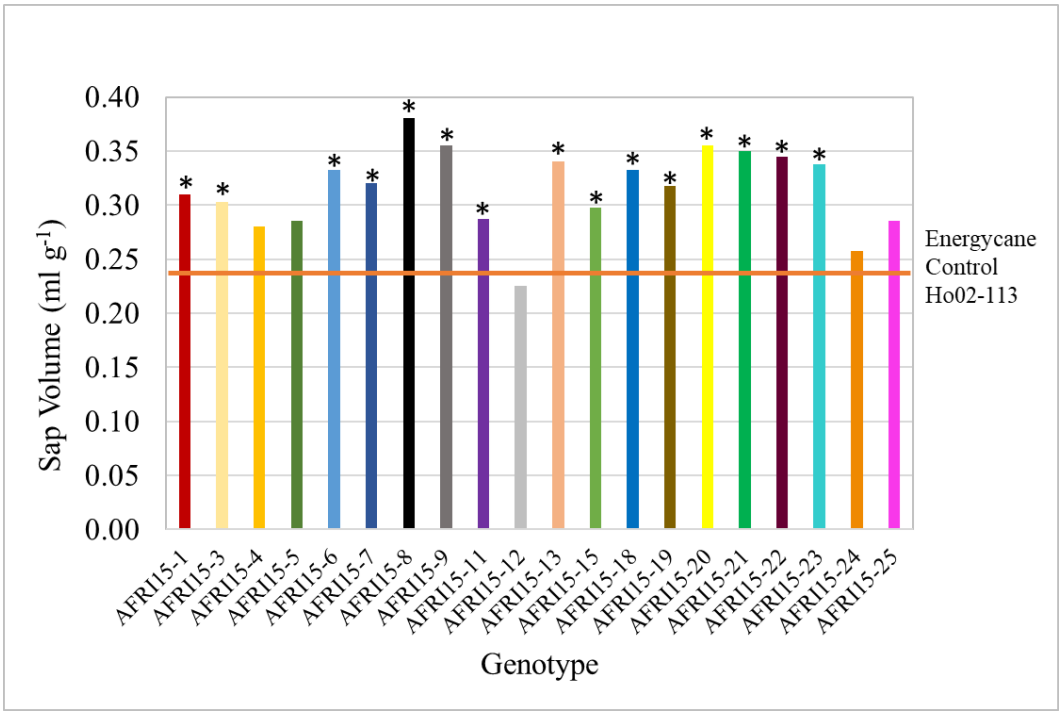

Figure 4.12 Mean sap volume yields in (ml g^{-1} fresh cane) for the plant cane year at the HH Leveck Animal Research Center in 2020. Black asterisk indicates genotype that was significantly greater in mean sap volume than the control (indicated by orange horizontal line). $P < 0.0001$. LSD = 0.0017 ml g^{-1}.

Sap volume production was significantly different among genotypes ($P < 0.0001$) at the Bearden Dairy Research Center in 2021 (Fig. 4.13). The mean SV across genotypes was 0.35 ml g^{-1}. The highest SV-yielding genotype was AFRI15-8 with a mean SV yield of 0.44 ml g^{-1}. The least mean SV was 0.25 L ha^{-1} (Ho 02-113). All genotypes produced significantly more SV g^{-1} than the control. There was an effect due to replication ($P = 0.0044$).

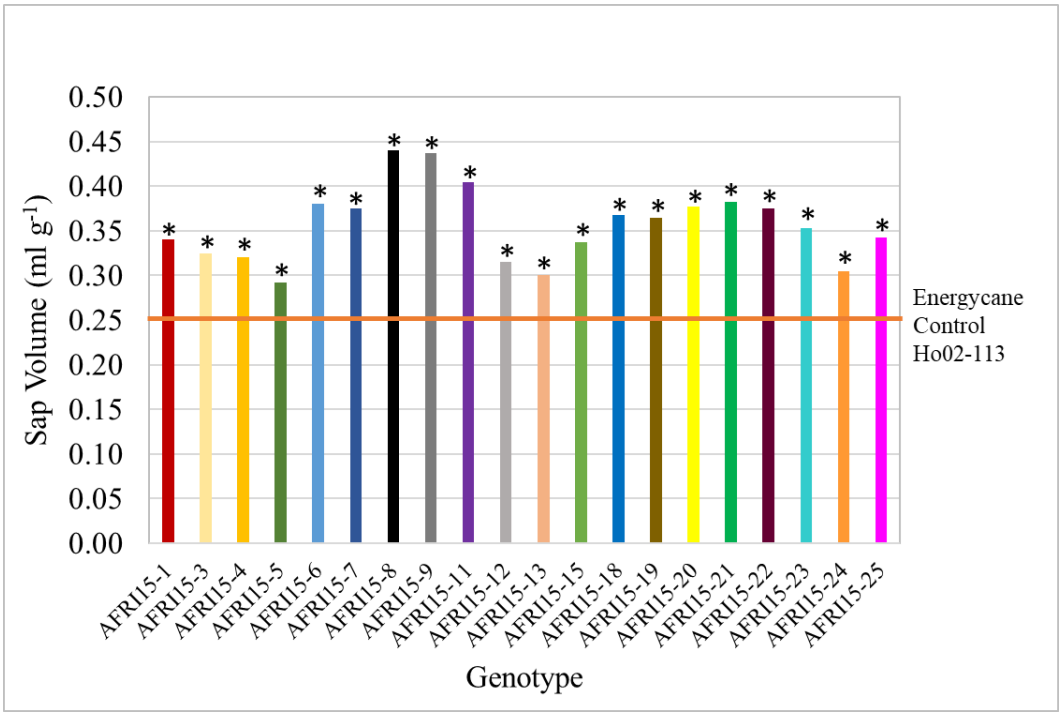

Figure 4.13 Mean sap volume yields (ml g^{-1} fresh cane) for the plant cane year at the Bearden Dairy Research Center in 2021. Black asterisk indicates genotype that was significantly greater in mean sap volume than the control (represented by the orange horizontal line). $P < 0.0001$. LSD = 0.05 ml g^{-1}.

There were significant differences in SV among genotypes ($P < 0.0001$) at the HH Leveck Animal Research Center in the first ratoon year (harvested in 2021; Fig. 4.14). The mean SV was 0.32 ml g^{-1}. The greatest SV was 0.41 ml g^{-1} from AFRI15-9. The least SV was 0.257 ml g^{-1} (AFRI15-5, AFRI15-24, and Ho 02-113). There were 15 genotypes with significantly greater SV than the control (indicated by black asterisks). There were no genotypes with

significantly less SV than the control. While there was a significant effect due to replication in the PC year, there was no effect due to replication in the ratoon year (P = 0.303).

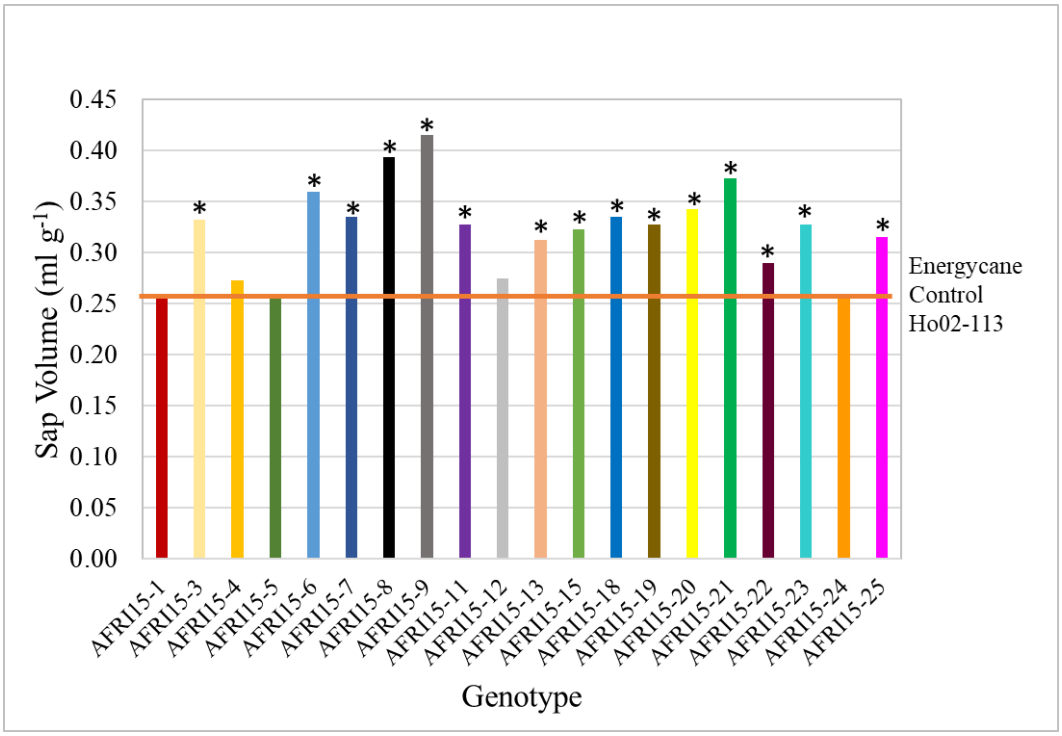

Figure 4.14 Mean sap volume yields (ml g^{-1} fresh cane) for the ratoon year at the HH Leveck Animal Research Center in 2021. Black asterisk indicates genotype that was significantly greater in mean sap volume than the control (represented by the orange horizontal line). P < 0.0001. LSD = 0.032 ml g^{-1}.

°Brix

°Brix is a measure of soluble carbohydrates in the sap. As such a simple multiplication of SV * °Brix can be used to estimate total yeast-fermentable ethanol production from a field of a particular genotype. There were significant differences in mean °Brix values (BV) among genotypes (P < 0.0217; Fig. 4.15). The mean BV across genotypes was 11.6. The greatest BV was 13.7 (AFRI15-1). The least BV was 8.9 (AFRI15-11). The mean BV for the control was

11.1. There were no genotypes with mean BV that were significantly different than the control. Mean BVs did not vary significantly among replications.

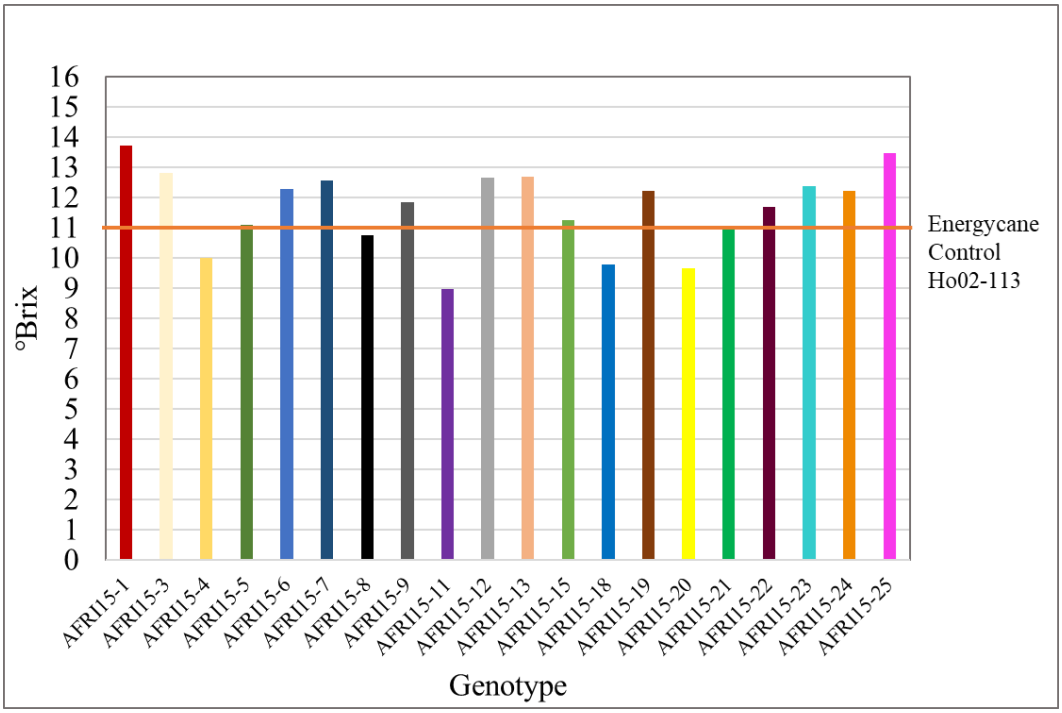

Figure 4.15 Mean °Brix values for the plant cane year at the HH Leveck Animal Research Center in 2020. No genotypes were significantly different from the control (represented by orange horizontal line). P = 0.0217. LSD = 2.6117.

During the repeat of the PC year at the Bearden Dairy Research Center (2021), significant differences in BV among genotypes ($P < 0.0001$) were observed (Fig. 4.16). The mean BV across genotypes was 12.61. The greatest BV was 15.55 (AFRI15-1). The least BV was 10.18 (AFRI15-18). The mean BV for the control was 12.15. There were four genotypes with significantly greater BV than the control (indicated by asterisks). No effect due to replications was observed ($P = 0.957$).

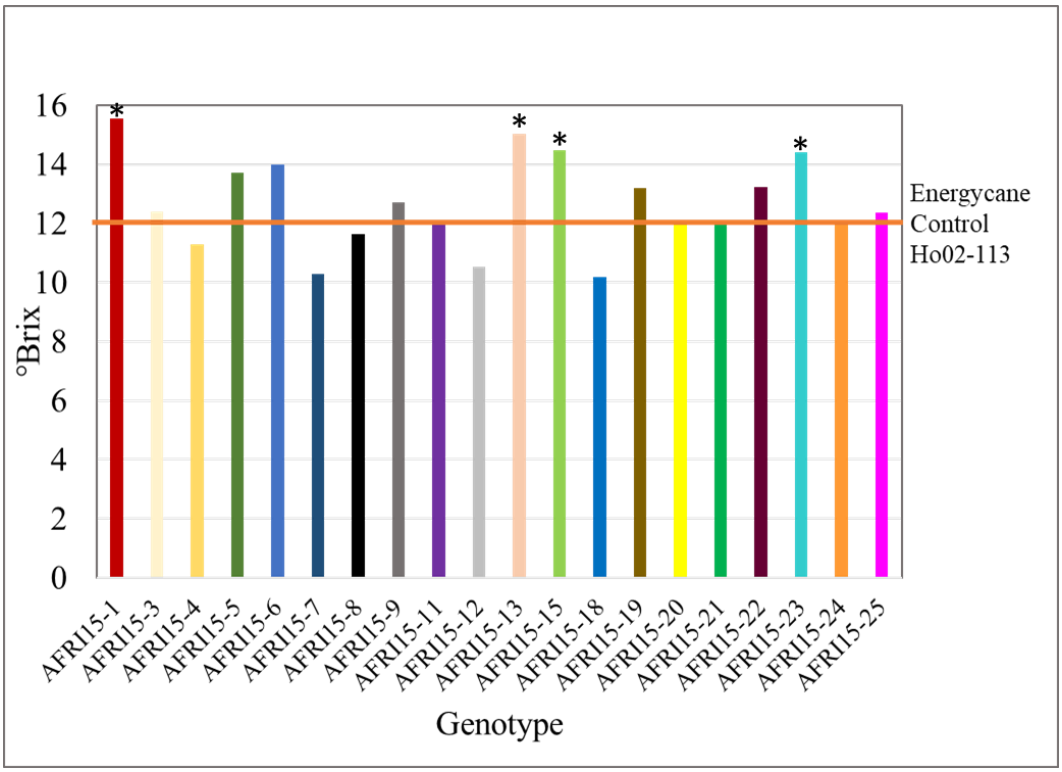

Figure 4.16 Mean °Brix values for the plant cane year at the Bearden Dairy research Center in 2021. Black asterisk indicates genotype with significantly greater mean °Brix values than the control (represented by orange horizontal line). $P < 0.0001$. LSD = 1.903

There were significant differences in BV among genotypes ($P < 0.0001$) at the HH Leveck Animal Research Center in 2021 (Fig. 4.17). The mean BV was 14.91. The greatest BV was 16.28 (AFRI15-22). The least BV was 12.78 (AFRI15-18). The mean BV for the control was 15.9. There were no genotypes with significantly greater BV than the control, but there were six genotypes with significantly lesser BV than the control (indicated by red asterisks). There was no effect due to replication ($P = 0.369$).

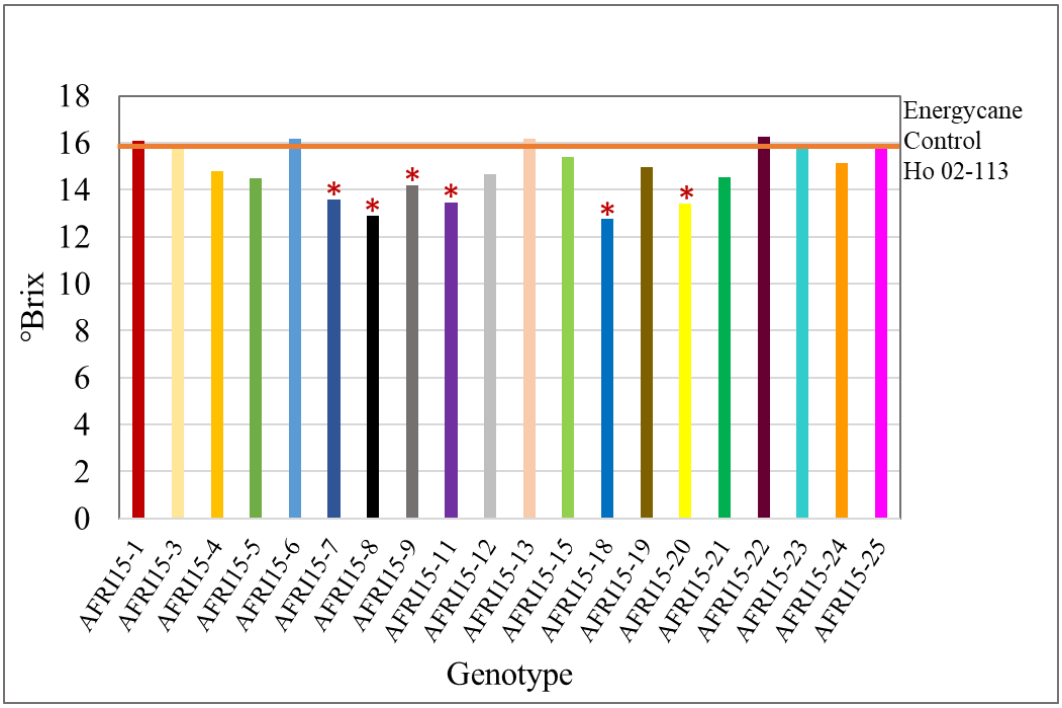

Figure 4.17 Mean °Brix values for ratoon year at HH Leveck Animal Research Center in 2021. Red asterisk indicates genotype with significantly lesser mean °Brix value than the control. P < 0.0001. LSD = 1.546.

Theoretical Ethanol from Sap

There were significant differences in theoretical ethanol from sap (TES) among genotypes in the PC year at the HH Leveck Animal Research Center in 2020 (Fig. 4.18), The mean TES among genotypes was 517.5 L ha^{-1}. The greatest mean TES was 791.13 L ha^{-1} (AFRI15-8). The least TES was 210.48 L ha^{-1} (AFRI15-4). The mean TES of the control was 235.51 L ha^{-1}. There were twelve genotypes with significantly greater TES than the control (indicated by black asterisks on Fig. 4.21). There were no genotypes with significantly lower TES than the control. There were significant differences in mean TES among replications (P < 0.0001). Theoretical ethanol from sap increased form the first replication (342.96 L ha^{-1}) to the fourth replication (671.82 L ha^{-1}).

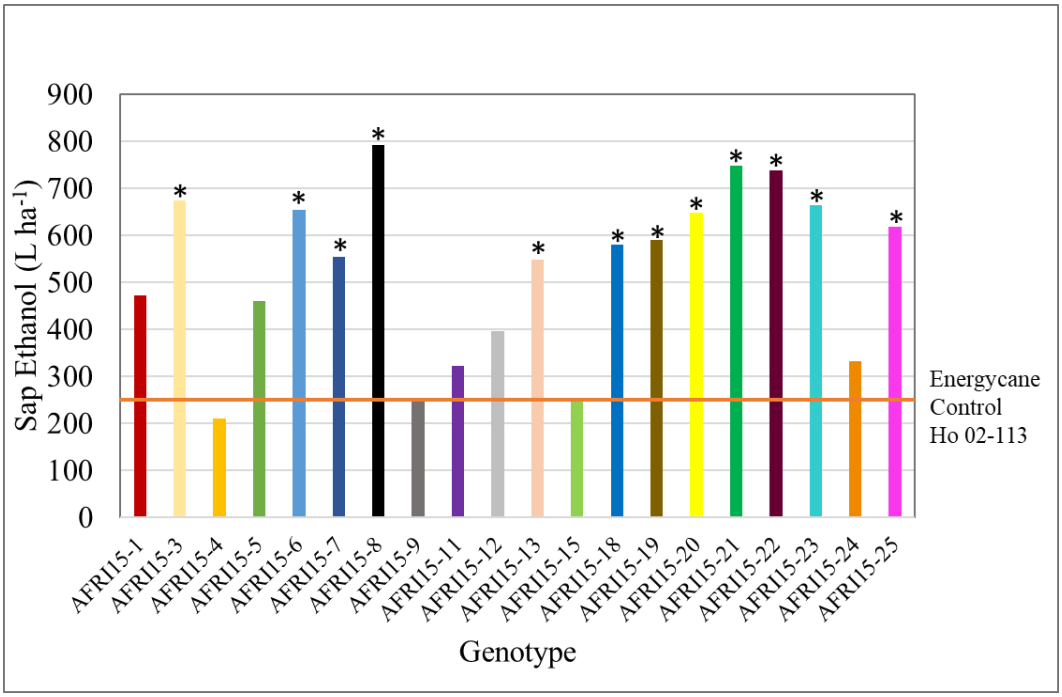

Figure 4.18 Mean theoretical ethanol production from sap (L ha^{-1}; Equation 1) for the plant cane year at the HH Leveck Animal Research Center in 2020. Black asterisk indicates genotype that was significantly greater in mean theoretical ethanol production from sap than the control (indicated by orange horizontal line). P < 0.0001. LSD = 277.94.

There were significant differences in theoretical TES among genotypes in the PC year at the Bearden Dairy Research Center in 2021 (P <0.0001; Fig. 4.19). The mean TES among genotypes was 1343.01 L ha^{-1}. The greatest mean TES was 2492.97 L ha^{-1} (AFRI15-9). The least TES was 805.79 L ha^{-1} (AFRI15-4). The mean TES of the control was 874.14 L ha^{-1}. There were seven genotypes with significantly greater TES than the control (indicated by black asterisks). There were no genotypes with significantly lesser TES than the control. There were no significant differences in mean TES among replications (P = 0.9175).

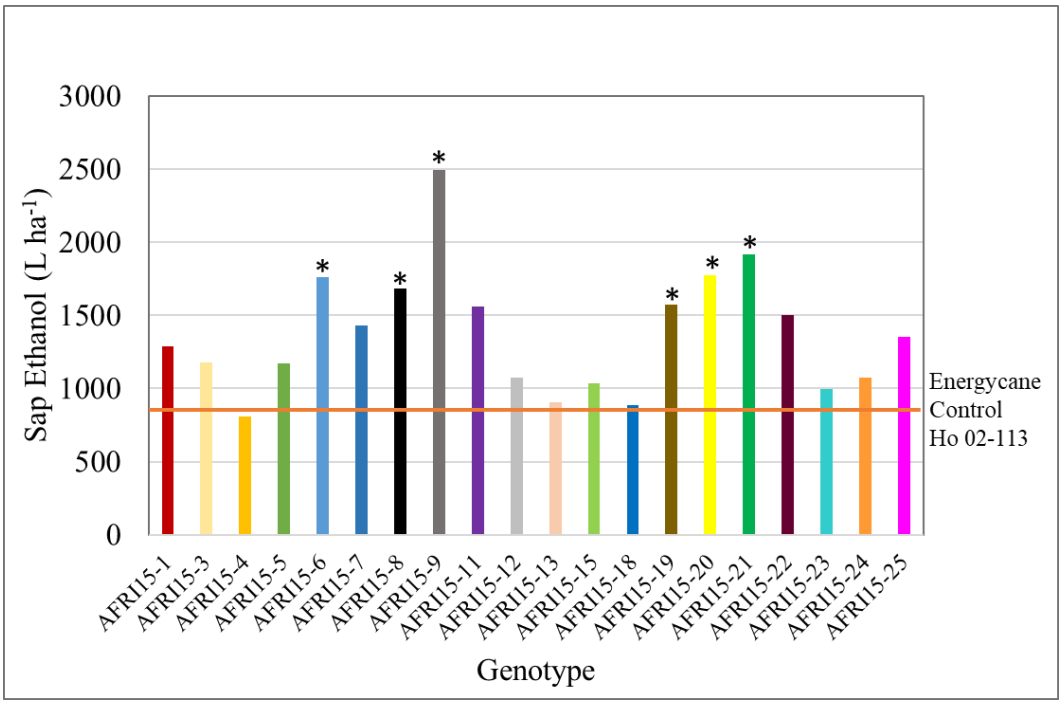

Figure 4.19 Mean theoretical ethanol production from sap (L ha^{-1}; Equation 1) for the Bearden Dairy Research Center in 2021. Black asterisks indicate genotypes that were significantly greater in mean theoretical ethanol from sap than the control (represented by the orange line). $P < 0.0001$. LSD = 609.49.

In the ratoon year at the HH Leveck Animal Research Center in 2021, there were significant differences in theoretical ethanol from sap (TES) among genotypes ($P < 0.0001$; Fig. 4.20). The mean TES among genotypes was 742.58 L ha^{-1}. The greatest mean TES was 1649.21 L ha^{-1} (AFRI15-3). The least TES was 51.32 L ha^{-1} (AFRI15-1). The mean TES of the control was 499.42 L ha^{-1}. There were three genotypes with significantly greater TES than the control (indicated by black asterisks), but Afri15-3 was not significantly greater than AFRI15-25. There were no genotypes with significantly lesser TES than the control. There was no effect in mean TES among replications ($P = 0.9175$).

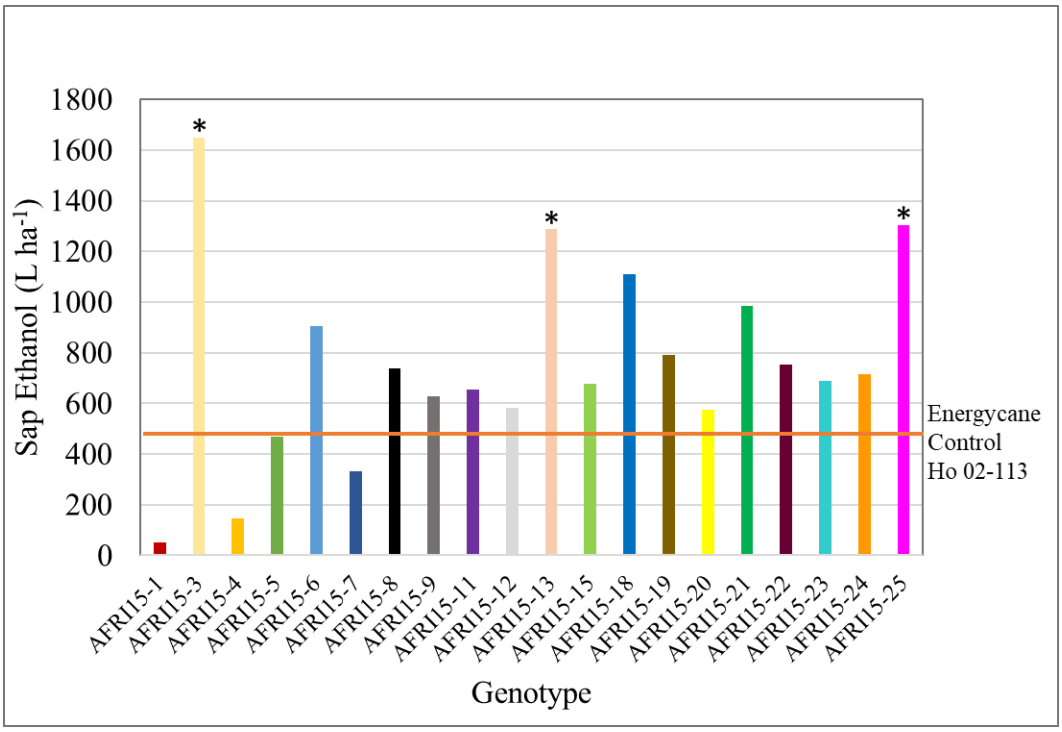

Figure 4.20 Mean theoretical ethanol production from sap (L ha^{-1}; Equation 1) for the HH Leveck Animal Research Center (2021). Black asterisks indicate genotypes that were significantly greater than the control. AFRI15-3 had the greatest theoretical ethanol. It was significantly greater than AFRI15-13, but it was not significantly greater than AFRI15-25. P = 0.0003. LSD = 619.71.

Theoretical Ethanol from Dry Matter

A constant value was multiplied by the dry matter yield to calculate theoretical ethanol from dry matter (TEDM; Dias et al., 2012). There were significant differences in TEDM among genotypes in the PC year at the HH Leveck Animal Research Center in 2020 (P <0.0025; Fig. 4.21). The mean TEDM among genotypes was 1934.31 L Mg^{-1} ha^{-1}. The greatest mean TEMD was 2643.75 L Mg^{-1} ha^{-1} (AFRI15-25). The least TEDM was 692.75 L Mg^{-1} ha^{-1} (AFRI15-1). The mean TEDM of the control was 2149.975 L Mg^{-1} ha^{-1}. There were no genotypes with significantly greater TEDM than the control, but there were four genotypes that were

significantly less than the control (indicated by red asterisks). There were significant differences among replications ($P < 0.0001$). The first replication had the least mean TEDM (1372.7 L Mg^{-1} ha^{-1}). The third replication had the greatest mean TEDM (2406.89 L Mg^{-1} ha^{-1}).

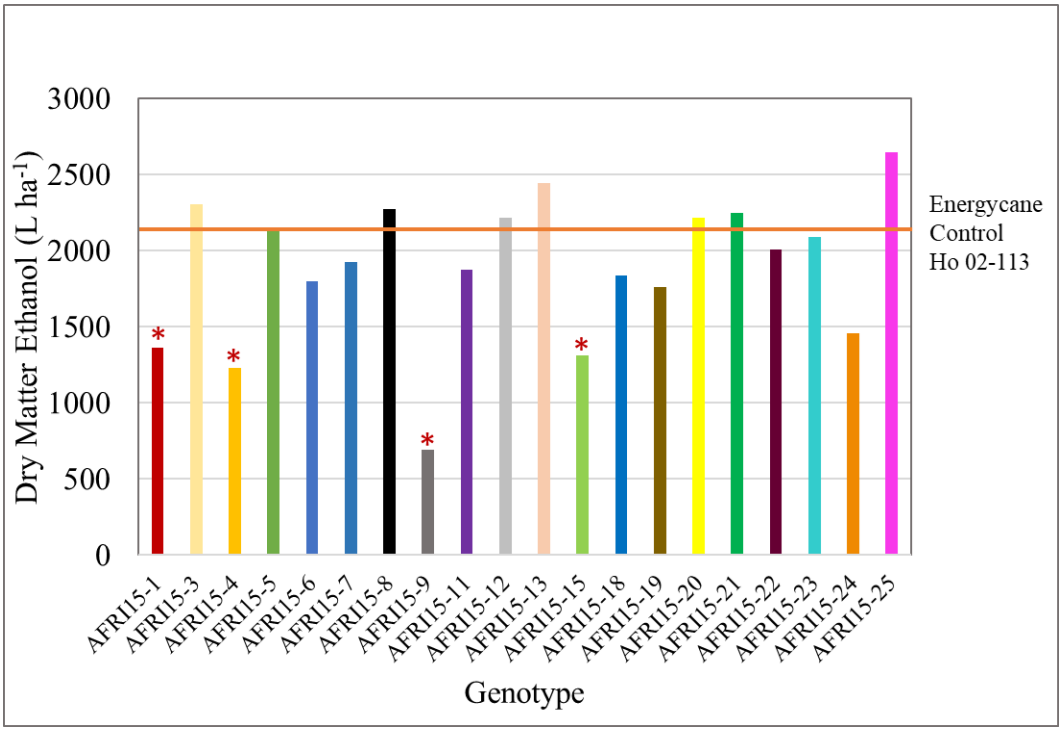

Figure 4.21 Mean theoretical ethanol from dry matter (L ha^{-1}; Equation 2) for the plant cane year at the HH Leveck Animal research Center in 2020. Red asterisk indicates genotype that was significantly lesser in mean theoretical ethanol from dry matter than the control (represented by orange horizontal line). P = 0.0025. LSD = 778.19.

There were significant differences in TEDM among genotypes in the PC year at the Bearden Dairy Research Center in 2021 ($P < 0.0026$; Fig. 4.22). The mean TEDM among genotypes was 2388.72 L Mg^{-1} ha^{-1}. The greatest mean TEMD was 2951.85 L Mg^{-1} ha^{-1} (AFRI15-7). The least TEDM was 1482.6 L Mg^{-1} ha^{-1} (AFRI15-23). The mean TEDM of the control was 2798.19 L Mg^{-1} ha^{-1}. There were no genotypes with significantly greater TEDM than

the control, but there were eight genotypes that were significantly lesser than the control (indicated by red asterisks). There were significant differences among replications ($P < 0.0155$). The first replication had the least mean TEDM (2121.2 L Mg^{-1} ha^{-1}). The second replication had the greatest mean TEDM (2580.4 L Mg^{-1} ha^{-1}).

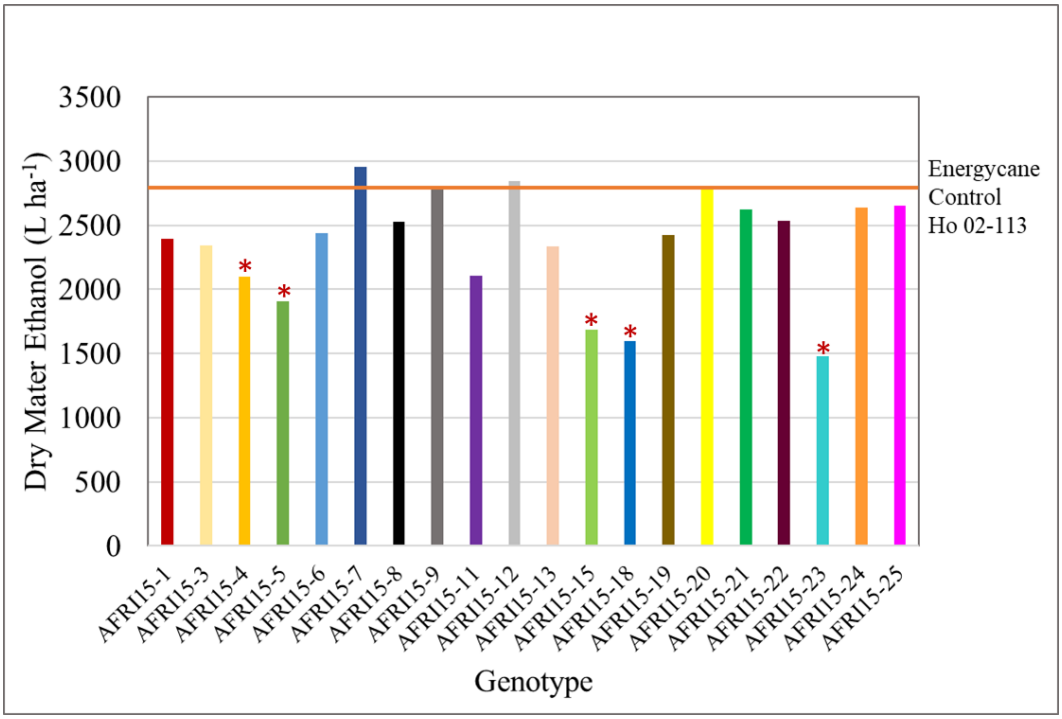

Figure 4.22 Mean theoretical ethanol from dry matter (L ha^{-1}; Equation 2) for the plant cane year at the Bearden Dairy Research Center in 2021. Red asterisk indicates genotype that was significantly lesser in mean theoretical ethanol from sap than the control (represented by the orange horizontal line). P = 0.0025. LSD = 734.38.

There were significant differences in theoretical ethanol from dry matter (TEDM) among genotypes in the ratoon year at the HH Leveck Animal Research Center in 2021 ($P < 0.0001$; Fig. 4.23). The mean TEDM among genotypes was 1637.38 L Mg^{-1} ha^{-1}. The greatest mean TEMD was 4121.4 L Mg^{-1} ha^{-1} (AFRI15-3). The least TEDM was 119.1 L Mg^{-1} ha^{-1}

(AFRI15-1). The mean TEDM of the control was 2164.6 L Mg^{-1} ha^{-1}. AFRI15-3 had a significantly greater TEDM than all other genotypes. There were three genotypes that were significantly lesser than the control (indicated by red asterisks). There were significant differences among replications (P = 0.0243). The second replication had the least mean TEDM (1319.4 L Mg^{-1} ha^{-1}). The third replication had the greatest mean TEDM (2053.4 L Mg^{-1} ha^{-1}).

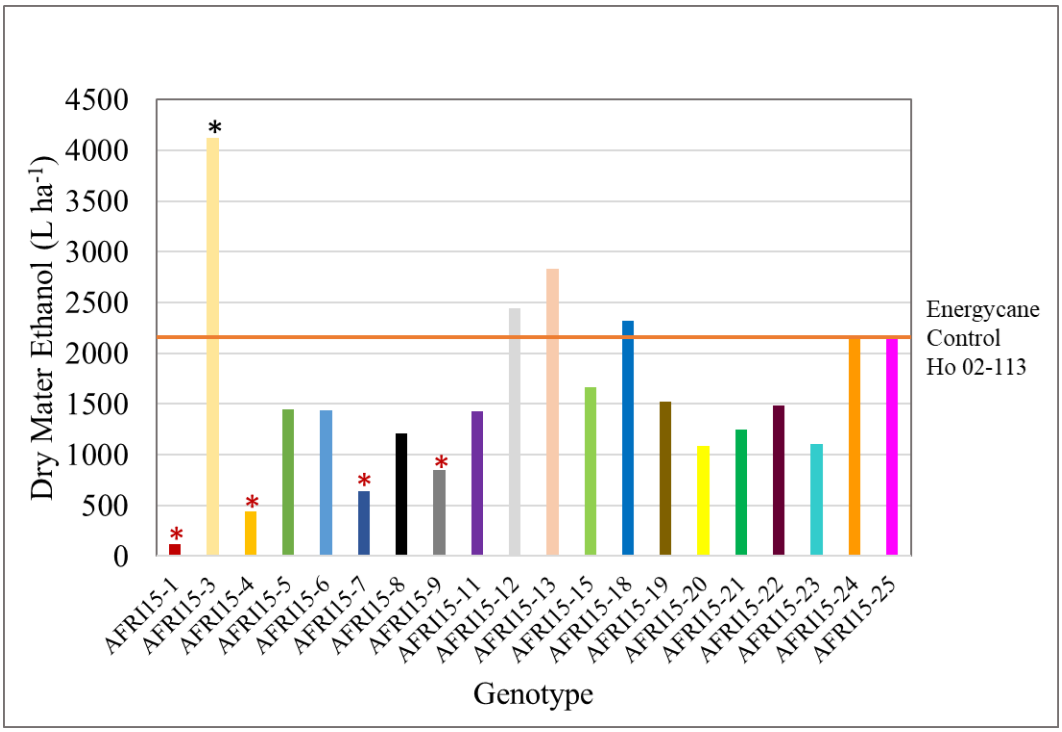

Figure 4.23 Mean theoretical ethanol from dry matter (L ha^{-1}; Equation 2) for the ratoon year at the HH Leveck Animal Research Center in 2021. AFRI15-3 had significantly greater TEDM than all other genotypes. Red asterisk indicates genotype that was significantly lesser in mean theoretical ethanol from sap than the control (represented by the orange horizontal line). P = 0.0001. LSD = 1163.6.

Total Theoretical Ethanol Yield

Total theoretical ethanol yield (TTEY) was calculated by adding the theoretical ethanol yield from the sap to the theoretical ethanol from the dry matter. The fractions are indicated

separately in figures 4.24, 4.25, and 4.26. There were significant differences in TTEY among genotypes in the PC year at the HH Leveck Animal Research Center in 2020 (P = 0.0011; Fig. 4.24). The mean TTEY among genotypes was 2451.82 L ha^{-1}. The greatest mean TTEY was 3261.22 L ha^{-1} (AFRI15-25). The least TTEY was 939.85 L ha^{-1} (AFRI15-9). The mean TEDM of the control was 2385.5 L ha^{-1}. There were no genotypes with significantly greater TTEY than the control; however, there was one genotype (indicated by red asterisk) which yield significantly less TTEY than the control. There were significant differences among replications (P < 0.0001). The first replication had the least mean TTEY (1715.65 L ha^{-1}). The fourth replication had the greatest mean TTEY (3078.7 L ha^{-1}).

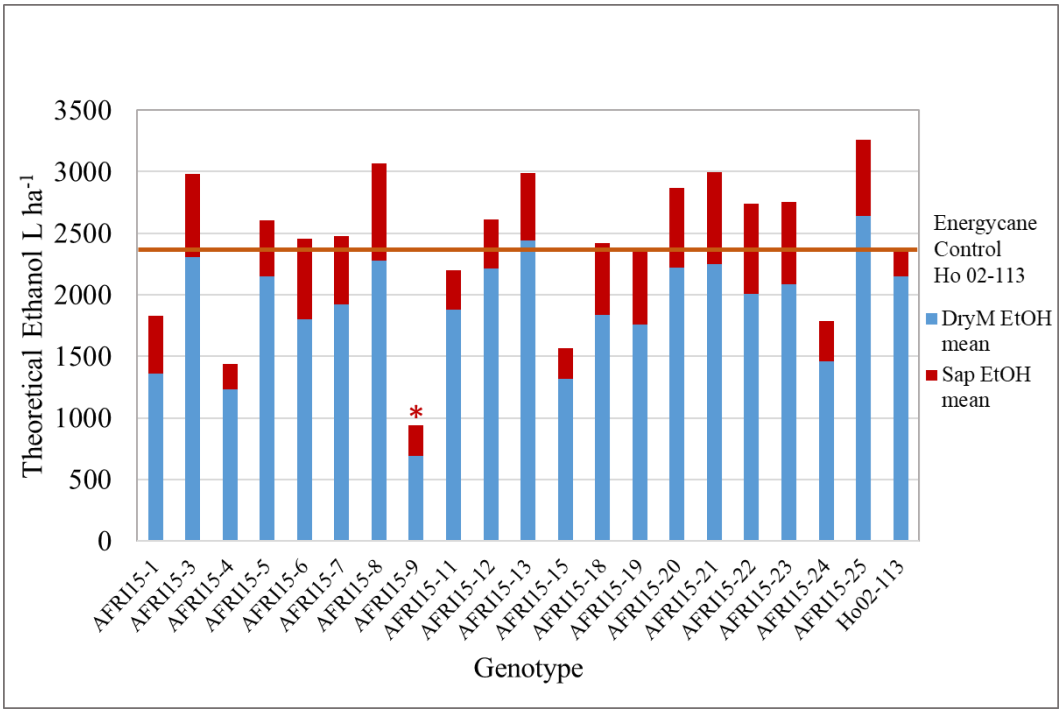

Figure 4.24 Mean total theoretical ethanol yield from plant cane year of HH Leveck Animal Research Center in 2020. Red asterisk indicates genotype that was significantly lesser in mean total theoretical ethanol than the control. P = 0.0011. LSD = 967.07.

There were significant differences in total theoretical ethanol yield (TTEY) among genotypes in the PC year at the HH Leveck Animal Research Center in 2021 (P = 0.0001; Fig. 4.25). The mean TTEY among genotypes was 3731.72 L ha^{-1}. The greatest mean TTEY was 5272.04 L ha^{-1} (AFRI15-9). The least TTEY was 2479.22 L ha^{-1} (AFRI15-23). The mean TTEY of the control was 3672.34 L ha^{-1}. There was one genotype (AFRI15-9, indicated by black asterisk) with significantly greater TTEY than the control; however, it was not significantly greater than AFRI15-20. There were two genotypes with significantly lesser TTEY than the control (indicated by red asterisks). There were no significant differences among replications (P = 0.1133).

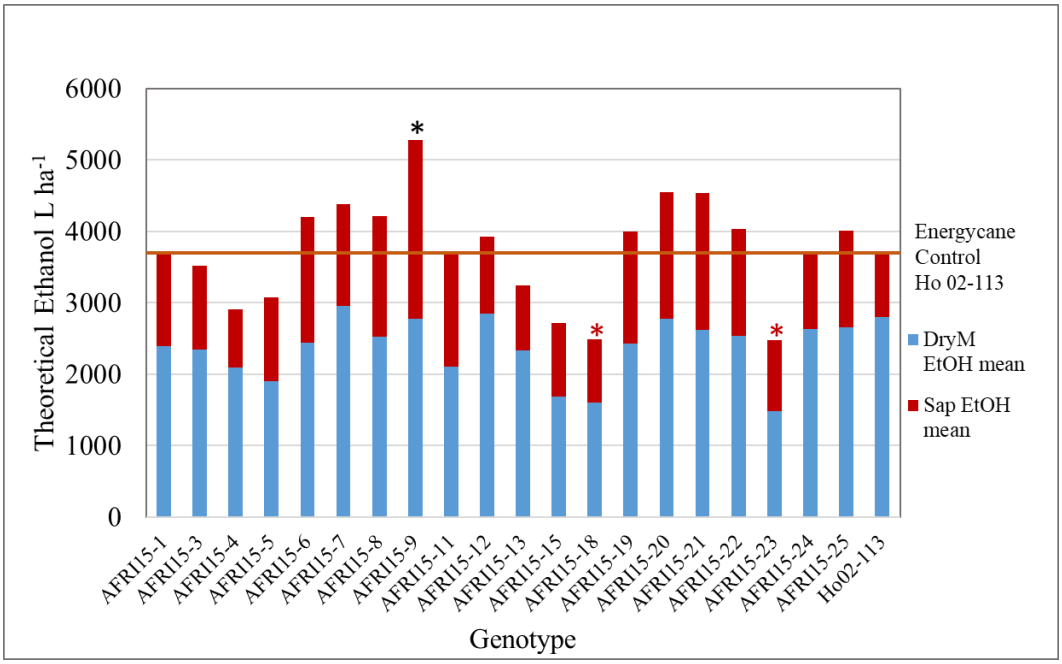

Figure 4.25 Mean total theoretical ethanol yield from plant cane year of Bearden Dairy Research Center in 2021. Black asterisk indicates genotype that was significantly greater than the control in mean total theoretical ethanol. Red asterisk indicates genotype that was significantly lesser than the control (represented by the orange horizontal line). P = 0.0001. LSD = 1085.1.

There were significant differences in total theoretical ethanol yield (TTEY) among genotypes in the ratoon year at the HH Leveck Animal Research Center in 2021 ($P < 0.0001$; Fig. 4.26). The mean TTEY among genotypes was 2379.96 L ha-1. The greatest mean TTEY was 5770.61 L ha-1 (AFRI15-3). The least TTEY was 170.45 L ha-1 (AFRI15-21). The mean TTEY of the control was 2664.04 L ha^{-1}. There was one genotype (AFRI15-3, indicated by black asterisk) with significantly greater TTEY than the control; however, it was not significantly greater than Afri15-13. There were three genotypes with significantly lesser TTEY than the control (indicated by red asterisks). There were significant differences among replications ($P = 0.0277$). The second replication had the least mean TTEY (1922.94 L ha^{-1}). The third replication had the greatest mean TTEY (2958.9 L ha^{-1}).

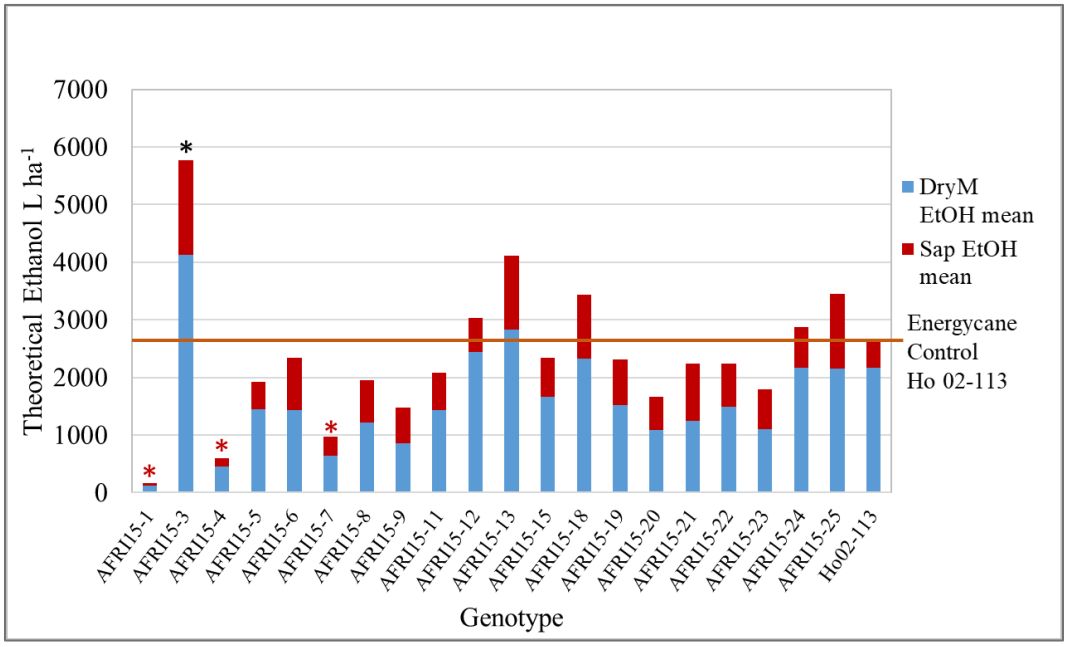

Figure 4.26 Mean total theoretical ethanol yield from ratoon year of HH Leveck Animal Research Center in 2021. AFRI15-3 was significantly greater than the control, but not AFRI15-13. Red asterisks indicate genotypes significantly lesser in TTEY than the control. $P < 0.0001$. LSD = 1665.8.

CHAPTER V

DISCUSSION

Comparison of Plant Cane Years Across Locations and Comparison of Plant Cane Year and Ratoon Year at HH Leveck Animal Research Center

Fresh Weight

Plant Cane Year Comparison

There was a significant difference between the PC year at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center in regard to FW ($P < 0.0001$; Table A.1). Mean FW was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center for most genotypes; however, there were two genotypes (AFRI15-5 and AFRI15-23) that decreased in FW from The HH Leveck Animal Research Center to the Bearden Dairy Research Center (Table A.2). The change in FW yield ranged from a decrease of 13.5% (AFRI15-23) to an increase of 315.7% (AFRI15-9). The relative percentage increase in FW for AFRI15-9 was due to the genotype's poor performance at the HH Leveck Animal Research Center; two replications failed to emerge, while the other two had very little emergence (see Fig. 5.1). This percentage increase was a common theme throughout the data, as AFRI15-9 performed very well at the Bearden Dairy Research Center. Two architectural characteristics are important to note in explanation of the percentage increase in FW from the HH Leveck Animal Research Center to the Bearden Dairy Research Center. In general, canes were taller at the Bearden Dairy Research Center. In general, for 18 of the 20 genotypes stand

densities were also greater at the Bearden Dairy Research Center than the HH Leveck Animal Research Center (see "Replication effects").

Plant Cane vs. Ratoon

There was a significant difference between the PC year and ratoon year at the HH Leveck Animal Research Center in regard to fresh weight ($P < 0.0001$; Table A.3). For the field as a whole, mean FW was significantly greater in the PC year than the ratoon year; however, while 15 genotypes declined in FW, there were five genotypes (AFRI15-3, AFRI15-9, AFRI15-15, AFRI15-18, and AFRI15-24) that increased in FW from the PC year to the ratoon year (Table A.4). The greatest percentage increase was 40.4% (AFRI15-3), and the greatest percentage decrease was 91.8% (AFRI15-1). In general, there was poor yield performance in the ratoon year of the HH Leveck Animal Research Center, particularly in the first two replications (Fig. 5.2). While there was less precipitation in the winter and spring in 2021 than in 2020, the poor drainage of the field likely exacerbated the damage from the freezing temperatures that year (see "Replication Effects"). It should be noted that the third replication of AFRI15-8 failed to emerge at all in the ratoon year. AFRI15-12 ranked among the greatest yielding genotypes for all three locations/years, indicating a strong genetic component to its respective yield.

Dry Matter Yield

Plant Cane Year Comparison

There was a significant difference between the PC year at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center in regard to DMY ($P < 0.0001$; Table A.5). Mean DMY was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center for most genotypes (Fig. 5.3); however, there were four

genotypes (AFRI15-5, AFRI15-13, AFRI15-18, and AFRI15-23) that decreased in DMY from HH Leveck Animal Research Center to the Bearden Dairy Animal Research Center (Table A.6). It should be noted that AFRI15-5 and AFRI15-23 decreased in DMY as in FW. Again, as with FW, the greatest percentage decrease was observed in AFRI15-23 (29.0%), and the greatest percentage increase was observed in AFRI15-9 (301.2%). These results are not surprising, being that FW and DM are highly correlated ($r^2 = 0.958$) in sugarcane production (Zhao et al., 2010).

Plant Cane vs. Ratoon Year

There was a significant difference between the PC year and ratoon year at the HH Leveck Animal Research Center in regard to DMY (P = 0.0008; Table A.7). Mean DMY was significantly greater in the PC year than the ratoon year; however, there were eight genotypes that did not follow the general trend. AFRI15-3, AFRI15-9, AFRI15-12, AFRI15-13, AFRI15-15, AFRI15-18, AFRI15 24, and Ho02-113 increased in DMY from the PC year to the ratoon year (Table A.8). The greatest percentage increase in DMY was 78.6% (AFRI15-3). The greatest percentage decrease in DMY was 91.3% (AFRI15-1). Seven genotypes decreased in DMY by 40% or greater in the ratoon year (AFRI15-1, AFRI15-4, AFRI15-7, AFRI15-8, AFRI15-20, AFRI15-21, and AFRI15-23). All the genotypes that increased in FWY also increased in DMY. However, two genotypes that decreased in FWY showed increases in DMY (AFRI15-12 and AFRI15-13). The decrease in FWY can be attributed to very poor emergence in one or more replications, but an increase in DMY in the other replications. The increase in fiber production in the successful replications was apparently able to make up for the losses in the other replications.

Sap Volume

Plant Cane Year Comparison

The characteristic that causes the difference between FW and DMY is the extractable sap volume. There was a significant difference between the PC year at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center in regard to SV production ($P < 0.0001$; Table A.9). Mean SV production was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center for most genotypes owing to the general greater yield at the Bearden Dairy Research Center (Table A.10). Percentage difference in SV ranged from an increase of 29.3% (AFRI15-22) to a decrease of 5% (Ho 02-113). The general increase in sap volume from the HH Leveck Animal Research Center to the Bearden Dairy Research Center can be attributed to the general increase in performance as previously mentioned.

Plant Cane vs. Ratoon

There was no significant difference between the PC year and ratoon year at the HH Leveck Animal Research Center in regard to sap volume production ($P = 0.1479$; Table A.11), as was observed with FW and DM. Thirteen genotypes increased in SV ml g^{-1} (AFRI15-3, AFRI15-6, AFRI15-7, AFRI15-8, AFRI15-9, AFRI15-11, AFRI15-12, AFRI15-15, AFRI15-18, AFRI15-19, AFRI15-21, AFRI15-25, and Ho 02-113; Table A.12). Percentage difference in SV range from an increase of 22.2% (AFRI15-12) to a decrease of 16.1% (AFRI15-1). There was an interaction effect between year and genotype ($P < 0.022$).

°Brix

Plant Cane Year Comparison

There was a significant difference observed between the PC year at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center in regard to BV ($P < 0.0001$; Table A.13). Mean BV was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center for most genotypes; however, there were five genotypes (AFRI15-3, AFRI15-7, AFRI15-12, AFRI15-24, and AFRI15-25) that decreased in BV from the HH Leveck Animal Research Center to the Bearden Dairy Research Center (Table A.14). The greatest percentage increase was 33.3% (AFRI15-11, an increase of 2.87° BV). The greatest percentage decrease was 18.2%, observed in AFRI15-7 (a decrease of 2.29° BV).

Plant Cane vs. Ratoon

There was a significant difference between the PC year and ratoon year at the HH Leveck Animal Research Center in regard to BV ($P <.0001$; Table A.15). Mean BV was significantly greater in the ratoon year than the PC year. All genotypes increased in BV in the ratoon year (Table A.16). The greatest percentage increase was 48% (AFRI15-4; increase of 4.8°). The least percentage increase was 8.1% (AFRI15-7; increase of 1.02°). An explanation of this may lie in the weather of the weeks leading up to harvest. The sample canes were taken on 26 Oct. 2021. The weeks preceding this date were very dry with no precipitation. Drier conditions in the ratoon year than in the PC year would have concentrated the sap volume as water transpiration losses were greater than root uptake (Figs. A.4 and A.5).

Theoretical Ethanol from Sap

Plant Cane Year Comparison

There was a significant difference between the PC years at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center in regard to TES ($P < 0.0001$; Table A.17). There were significant differences among genotypes in regard to TES due to differences in SV and BV. Mean TES value was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center. Theoretical ethanol from sap increased among all genotypes from the HH Leveck Animal Research Center to the Bearden Dairy Research Center (Table A.18). There was a significant interaction between genotype and location ($P = 0.0431$). The percentage increase in TES is not a surprise since all genotypes increased in SV from the HH Leveck Animal Research Center to the Bearden Dairy Research Center. Sap volume per unit land is, in this case, a more important driver of TES than the BV, since not all genotypes increased in BV at the Bearden Dairy Research Center.

Plant Cane vs Ratoon

There was a significant difference between the PC year and the ratoon year at the HH Leveck Animal Research Center in regard to TES ($P < 0.0001$; Table A.19). Even though FW declined from PC to ratoon year, mean TES was significantly greater in the ratoon year than in the PC year. There were significant differences among genotypes in regard to TES ($P < 0.0001$). The greatest percentage increase in TES was 144.7% (AFRI15-3; Table A.20). The greatest percentage decrease in TES was 89.1% (AFRI15-1). Five genotypes decreased in TES in the ratoon year (AFRI15-1, AFRI15-4, AFRI15-7, and AFRI15-8). These decreases are likely due to significant decreases in total amount of cane (see DMY) in the ratoon year. The increases in TES can be attributed to both the increase volume due to the increase in height of the random sample

canes, as well as the increase in BVs (mean percentage increase of 28.5%; Table A.16) from the PC year to the ratoon year.

Theoretical Ethanol from Dry Matter

Plant Cane Year Comparison

There was a significant difference between the PC years at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center in regard to TEDM ($P < 0.0001$; Table A.21). There were significant differences among genotypes in regard to TEDM. Mean TEDM value was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center; although, four genotypes decreased in TEDM from HH Leveck Animal Research Center to the Bearden Dairy Research Center (Table A.22). There was an effect on the mean due to replication ($P < 0.0001$). The relationship among genotypes follows DMY exactly. The greatest percentage increase was AFRI15-9 (301.2%), and the greatest percentage decrease was AFRI15-23 (29.0%).

Plant Cane vs Ratoon

There was a significant difference between the PC year and the ratoon year at the HH Leveck Animal Research Center in regard to theoretical ethanol from dry matter ($P < 0.0001$; Table A.23). There were significant differences among genotypes in regard to TEDM ($P < 0.0001$). Mean TEDM value was significantly greater in the PC year than the ratoon year at the HH Leveck Animal Research Center (Table A.24). The greatest percentage increase in TEDM was 78.6% (AFRI15-3). The greatest percentage decrease in TEDM was 91.2% (AFRI15-1). Again, these changes follow DMY. There was a significant effect due to replication ($P < 0.0001$) and a significant interaction between genotype and replication ($P = 0.0001$).

Total Theoretical Ethanol Yield

Plant Cane Year Comparison

There was a significant difference in total theoretical ethanol yield (TTEY) between the PC years at the HH Leveck Animal Research Center and the Bearden Dairy Animal Research Center ($P < 0.0001$; Table A.25). There were significant differences among genotypes in regard to TTEY ($P = 0.0096$). The mean TTEY value was significantly greater at the Bearden Dairy Research Center than at the HH Leveck Animal Research Center owing to the general increased growth at the Bearden Dairy Research Center. All genotypes (with the exception of AFRI15-23) increased in TTEY from the HH Leveck Animal research Center to the Bearden Dairy Research Center (Table A.26). There was significant effect due to replication ($P < 0.0001$). The greatest percentage increase from the HH Leveck Animal Research Center to the Bearden dairy Research Center was AFRI15-9 (460.9%). The greatest percentage decrease was AFRI15-23 (10%).

Plant Cane vs Ratoon

There were significant differences among genotypes in regard to TTEY ($P < 0.0001$), but there was no significant difference between the PC year and the ratoon year at the HH Leveck Animal Research Center ($P = 0.5866$; Table A.27). There was a significant effect due to replication ($P < 0.0001$) and significant interaction between genotype and replication ($P = 0.0003$). The greatest percentage increase in TTEY was 93.6% AFRI15-3; Table A.28). The greatest percentage decrease in TTEY was 90.7% (AFRI15-1). The trends in TTEY follow more closely with the DMY than the TES. This is not surprising, given that energycane are less valuable as a sugar crop in favor of fiber production. It is important to note that DMY is a stronger indicator of TTEY than SV and BV. As mentioned previously, the general decline in

TTEY, which is reflected in DMY, is likely due to poor drainage in the field, which was exacerbated by the freezing temperatures during the dormant period.

Replication Effects

Replication effects were present in both PC locations as well as the ratoon year at the HH Leveck Animal Research Center (as mentioned previously). It appears that some genotypes are highly sensitive to environmental effects. Using Microsoft® Excel, a color scale format was applied to the maps in order to visualize variance in the fields. The dry weights per plot in kilograms were listed in the cells. Darker colors indicate greater yields; lighter colors indicate lower yields. Figure 5.1 shows great variability across the field in the PC year of the HH Leveck Animal Research Center.

BORDER			BORDER			BORDER			BORDER		
14.5	23.9	16.4	22.9	2.0	3.5	28.4	15.8	22.1	38.6	30.3	44.2
22.6	23.9	20.3	14.4	9.0	17.5	33.2	30.3	37.7	32.6	26.2	24.2
15.2	19.4	11.0	29.8	17.2	15.5	18.2	29.3	27.7	29.3	27.0	28.0
21.4	20.9	12.4	18.2	28.2	27.9	48.4	31.0	29.4	39.5	21.5	26.4
18.7	23.4	2.2	31.8	22.9	25.6	35.6	40.9	43.7	35.4	38.3	34.8
12.8	19.8	.	10.3	27.7	23.2	34.1	37.3	31.1	.	19.7	30.9
24.6	2.0	25.7	9.0	30.3	36.7	10.4	25.6	29.2	31.7	23.1	34.4
BORDER			BORDER			BORDER			BORDER		
REOLICATION 1			REPLICATION 2			REPLICATION 3			REPLICATION 4		

Figure 5.1 Yield map of plant cane year (2020) at the HH Leveck Animal Research Center demonstrating the replication effect. Numbers are the dry matter yield in kg plot^{-1}. The color scale format in Microsoft® Excel assigns deeper color to greater values. The two cells represented by a period are both plots of AFRI15-9 that failed to emerge.

Some environmental effect appears to have caused the significant differences in replication. No plot in the first replication exceeded 25.7 kg. There were two missing data points

represented by a period. These two missing points were both plots of AFRI15-9, which never emerged in the first or fourth replication. This environmental effect seems to have caused the replication effect and was especially detrimental to AFRI15-9. This effect was exacerbated in the ratoon year, as Figure 5.2 demonstrates. It is likely, as mentioned previously, that the excessive rainfall in 2020 and poor drainage in the field were to blame.

BORDER			BORDER			BORDER			BORDER		
29.0	15.9	6.9	25.2	5.5	1.0	22.9	16.3	20.1	5.5	43.5	45.9
28.2	20.4	14.2	17.2	0.6	1.5	31.5	61.5	12.9	14.3	19.9	17.1
37.7	9.9	5.2	22.8	7.7	2.9	1.9	25.8	14.5	15.7	11.7	37.3
20.9	1.3	10.9	7.7	13.3	16.4	39.0	33.4	28.0	46.3	2.4	5.4
27.9	32.5	3.7	58.8	26.5	20.4	40.3	20.5	48.5	40.2	10.6	26.3
9.7	0.8	.	11.4	25.0	17.9	46.3	7.2	.	.	16.3	41.7
41.9	5.7	47.2	27.4	14.3	31.2	5.3	33.5	16.2	18.7	16.6	12.0
BORDER			BORDER			BORDER			BORDER		
REPLICATION 1			REPLICATION 2			REPLICATION 3			REPLICATION 4		

Figure 5.2 Yield map of ratoon year (2021) at the HH Leveck Animal Research Center demonstrating the replication effect. Numbers are the dry matter yield in kg plot^{-1}. The color scale format in Microsoft® Excel assigns deeper color to greater values. The three cells represented by a period are plots of AFRI15-9 (replication 1 and replication 4) and AFRI15-8 (replication 3) that failed to emerge.

At the Bearden Dairy Research Center, there were also replication effects in regard to fresh weights and dry matter yields (as mentioned previously). Figure 5.3 demonstrates the variability across the field. Two data points are missing (represented by a period). One due to missing data, the other due to the plot never having been planted. The first replication had the lowest yields.

BORDER		BORDER	
25.7	34.7	24.5	19.8
31.0	25.1	38.2	23.5
.	34.5	23.9	22.9
26.4	39.9	29.0	33.2
16.3	38.4	37.4	18.9
24.2	27.6	35.5	36.9
21.1	35.3	28.0	.
33.1	40.1	33.0	28.5
26.3	34.9	17.4	24.7
19.7	32.6	35.4	35.7
36.2	15.3	39.9	27.6
27.0	21.5	34.2	29.0
27.2	27.1	39.9	20.8
24.0	32.0	21.6	21.8
28.4	29.3	35.6	38.5
22.5	32.6	34.6	26.5
40.9	34.9	22.0	38.2
36.2	43.4	39.5	45.1
34.1	45.1	43.5	18.9
32.4	38.2	41.8	37.1
35.6	31.0	30.7	40.7
BORDER		BORDER	
REP 1	REP 2	REP 3	REP 4

Figure 5.3 Dry matter yield map at the Bearden Dairy Research Center (2021) demonstrates replication effect and variability across field. Numbers are the dry matter yield in kg plot^{-1}. The color scale format in Microsoft® Excel assigns deeper color to greater values. The cells represented by a period in rep 1 and rep 4 are due to missing data.

The environmental effects across fields could be due to drainage issues. From 1 Jan. 2020 to 30 April 2020, during the PC year at the HH Leveck Animal Research Center, Starkville, MS received 111 cm of precipitation, whereas, in 2021 for the same period, only 39 cm of precipitation fell (*Daily Report - Delta Agricultural Weather Center* 2022). Drain tiles had also been installed in the field to increase drainage; however, sometime in the recent past, some of those drain tiles were broken (Baldwin, personal communication). This could also explain the significantly lower yields at the HH Leveck Animal Research Center. Freezing weather was not

a factor which harmed yields in the PC year at the HH Leveck Animal Research Center in 2020. This is because in 2021, the temperature remained at or below freezing for six days between 13 Feb. 2021 to 18 Feb. 2021 (*Daily Report - Delta Agricultural Weather Center* 2022). Despite this atypical freezing weather for Starkville, MS, the PC year at the Bearden Dairy Research Center yielded significantly greater than the HH Leveck Animal Research Center. This freezing weather coupled with the poor drainage may have exacerbated the environmental effect at the HH Leveck Animal Research Center and caused the decrease in DMY in many of the genotypes

CHAPTER VI

SUMMARY AND CONCLUSIONS

Evaluating Performance of Genotypes

When considering a feedstock for cellulosic conversion, biomass quantity is more important than quality (Sanford et al., 2017). Subscribing to this, dry matter yield would be the most important tool for estimation of fuel value. When evaluating the genotypes for bioenergy in North-Central MS, DMY in Tables 7.7 and 7.9 were ordered from greatest to least yield according to location and year. Table 6.1 is a comparison of the top ten genotypes during their respective PC years. The genotypes marked with an asterisk are those that yielded greatest at both locations. While Table 6.1 demonstrates the greatest yielding in their PC year, this does not tell which genotypes are best-suited. Table 6.2 shows these same genotypes but also the percentage difference between DMY (Mg ha^{-1}) at the two locations.

Table 6.1 Ten greatest yielding genotypes at both plant cane locations.

PC Year HH Leveck Animal Research	PC Year Bearden Dairy Research Center
AFRI15-25*	AFRI15-7
AFRI15-13	AFRI15-12*
AFRI15-3	Ho02-113*
AFRI15-8*	AFRI15-9
AFRI15-21*	AFRI15-20*
AFRI15-20*	AFRI15-25*
AFRI15-12*	AFRI15-24
Ho02-113*	AFRI15-21*
AFRI15-5	AFRI15-22
AFRI15-23	AFRI15-8*

Asterisk indicates genotype that yielded in top ten in both plant cane locations.

Table 6.2 Top yielding varieties that occur in both plant cane locations and percentage difference between locations.

Genotype	PC Year HH Leveck Animal Research	PC Year Bearden Dairy Research Center	Percentage Difference
AFRI15-25	15.1	15.2	0.2
AFRI15-8	13.0	14.5	11.0
AFRI15-21	12.9	15.1	16.6
AFRI15-20	12.7	16.0	25.3
AFRI15-12	12.7	16.3	28.4
Ho02-113	12.3	16.1	30.2

The difference in DMY for AFRI15-25 between the first location and the second location was less than one percent indicating consistency in yield across the two very different environments. This would indicate the genetics of specific genotypes was able to overcome the environmental effects between locations. From these two tables, we can conclude that AFRI25-25 performed the most consistently in the PC year.

The ability to ratoon is essential to producing feedstocks that are both renewable and sustainable. Table 6.3 compares the top ten yielding genotypes during the PC year and the ratoon year at the HH Leveck Animal Research Center.

Table 6.3 Top ten yielding genotypes during both plant cane year and ratoon year at the HH Leveck Animal Research Center.

PC Year HH Leveck Animal Research	**Ratoon Year HH Leveck Animal Research Center**
AFRI15-25*	AFRI15-3*
AFRI15-13*	AFRI15-13*
AFRI15-3*	AFRI15-12*
AFRI15-8	AFRI15-18
AFRI15-21	Ho02-113*
AFRI15-20	AFRI15-24
AFRI15-12*	AFRI15-25*
Ho02-113*	AFRI15-15
AFRI15-5	AFRI15-19
AFRI15-23	AFRI15-22

Asterisks indicate varieties that yielded in top ten in both years.

Table 6.4 demonstrates those genotypes that ranked in the top ten based on yield in both locations with the percentage difference between the PC year and the ratoon year.

Table 6.4 Top yielding genotypes that occur in both plant cane locations and percentage difference.

Genotype	PC Year HH Leveck Animal Research	Ratoon Year HH Leveck Animal Research	Percentage Difference
AFRI15-25	15.1	12.3	-18.7
AFRI15-13	13.0	16.2	15.7
AFRI15-3	12.9	23.7	78.7
AFRI15-12	12.7	14.0	10.3
Ho02-113	12.7	12.4	0.7

While AFRI15-25 yielded high in the PC years, and above the mean in the ratoon year, it is clearly in decline with respect to yield. AFRI15-3, however, did not yield in the top ten in both locations, but increased in the ratoon year by 78%. AFRI15-12 yielded in the top ten in both PC year and increased in DMY during the ratoon year.

Bioenergy crops need to be sustainable. *Saccharum* is relatively new to 33°N latitude. High yields, consistency in ratoon years, and consistency across environments are all important in the production of a biomass crop that will not compete for land with food crops. Within the limitations of this study, AFRI15-3 performed the best in the ratoon year, having increased in yield in an environment that was not conducive for most of the other genotypes. Although AFRI15-3 was not among the top ten at the Bearden Dairy Research Center, it is important to note that its DMY between the two locations during the PC year were within two percent of each other (Table 7.7). This consistency further indicates a genotype's value as a potential for further

testing and breeding efforts. Other yield-stable genotypes include AFRI15-12, AFRI15-25, and Ho 02-113, all of which yielded in the top ten in both years at the HH Leveck Animal Research Center (although DMY for AFRI15-25 is clearly in decline). Further testing is needed to determine genotypes that are best suited for biomass production in Northcentral Mississippi.

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APPENDIX A

FIGURES AND TABLES

Mean Height Tables and Weather Data

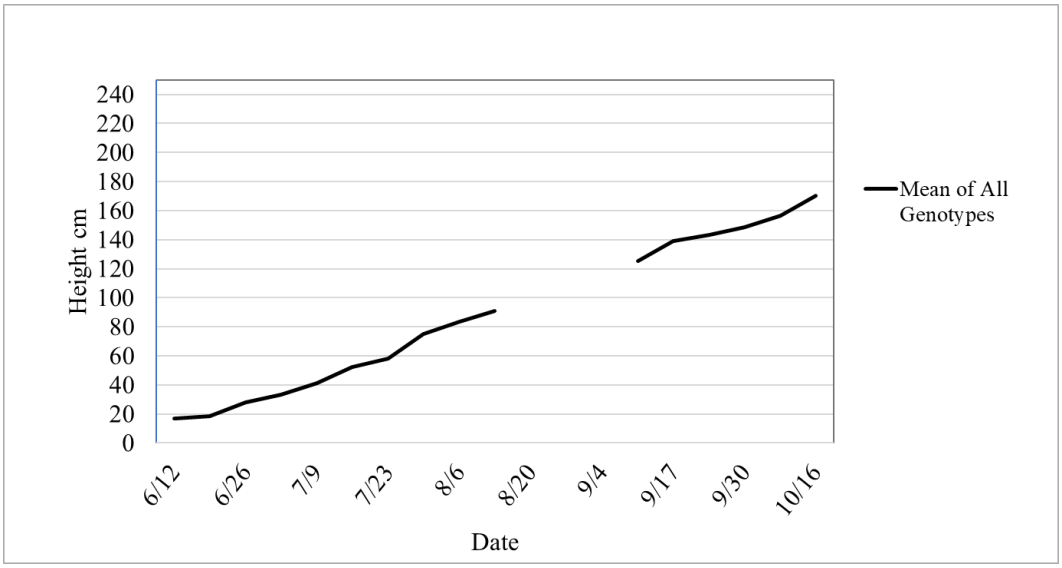

Figure A.1 Mean height of all genotypes at HH Leveck Animal Research Center during PC year (2020). Gap in data from 20 Aug. 2020 to 11 Sept. 2020 were due Covid-19 quarantines.

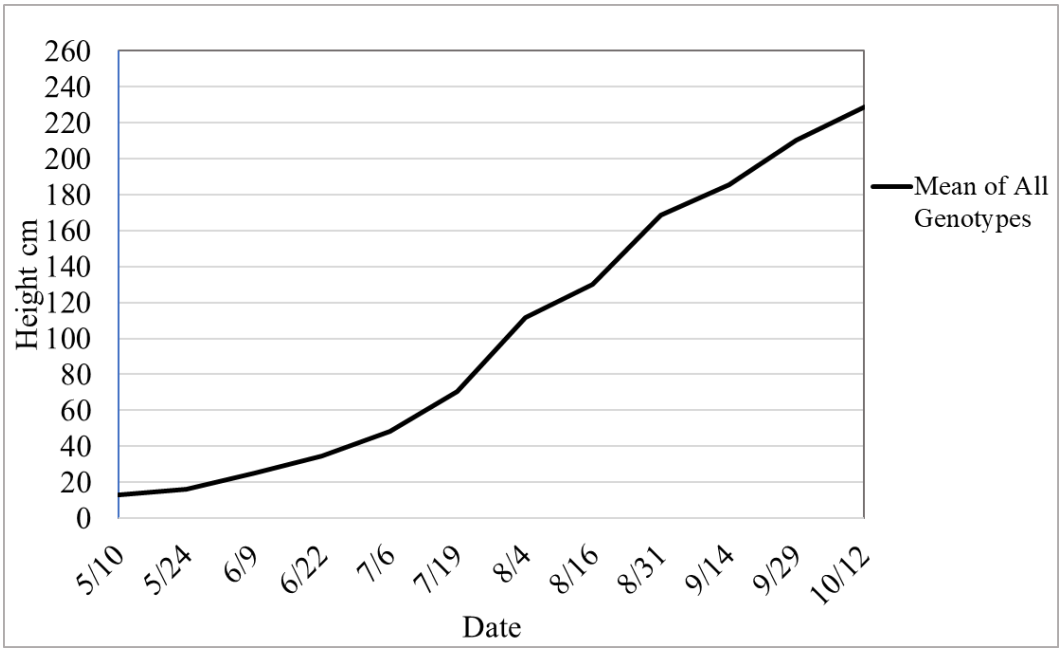

Figure A.2 Mean height of all genotypes at the Bearden Dairy Research Center during PC year (2021).

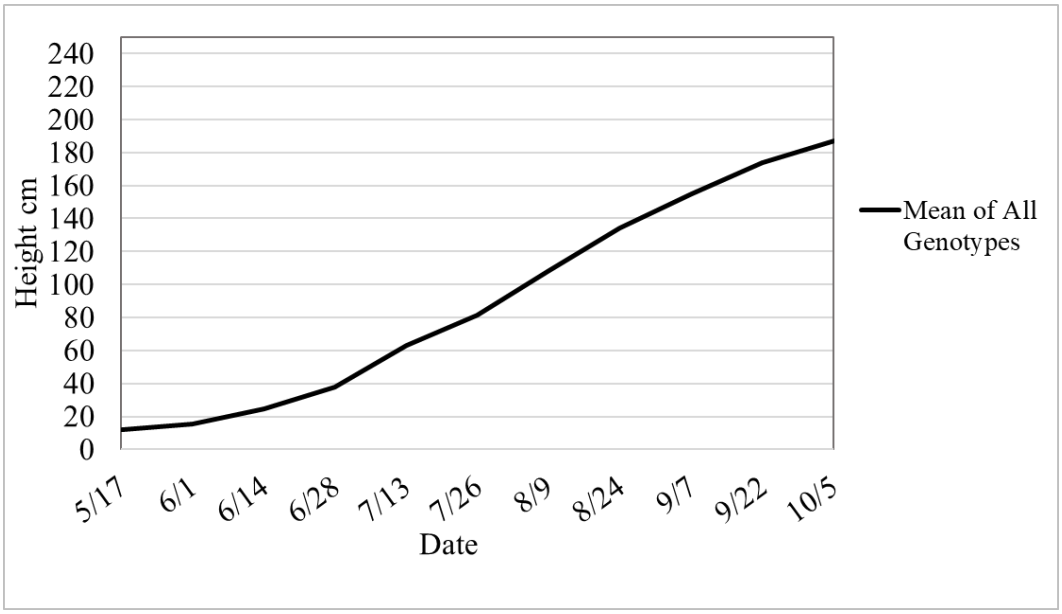

Figure A.3 Mean height of all genotypes at the HH Leveck Animal research Center during the ratoon year (2021).

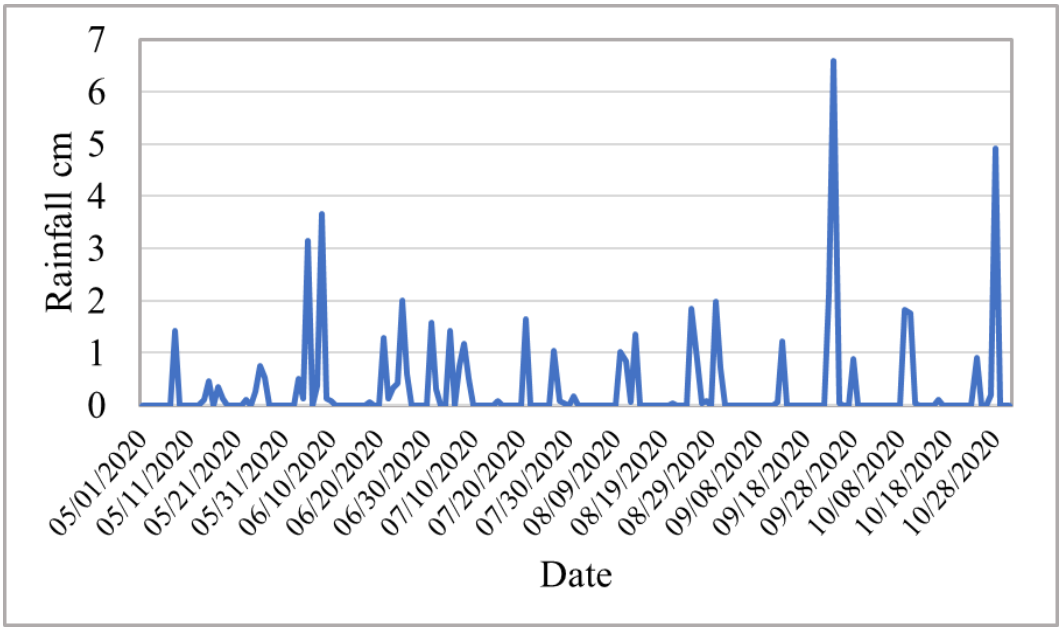

Figure A.4 Daily rainfall in cm from 1 May 2020 to 31 Oct. 2020 recorded at the R.R. Foil Plant Science Research Center in Starkville, MS. Total rainfall was 55.14 cm.

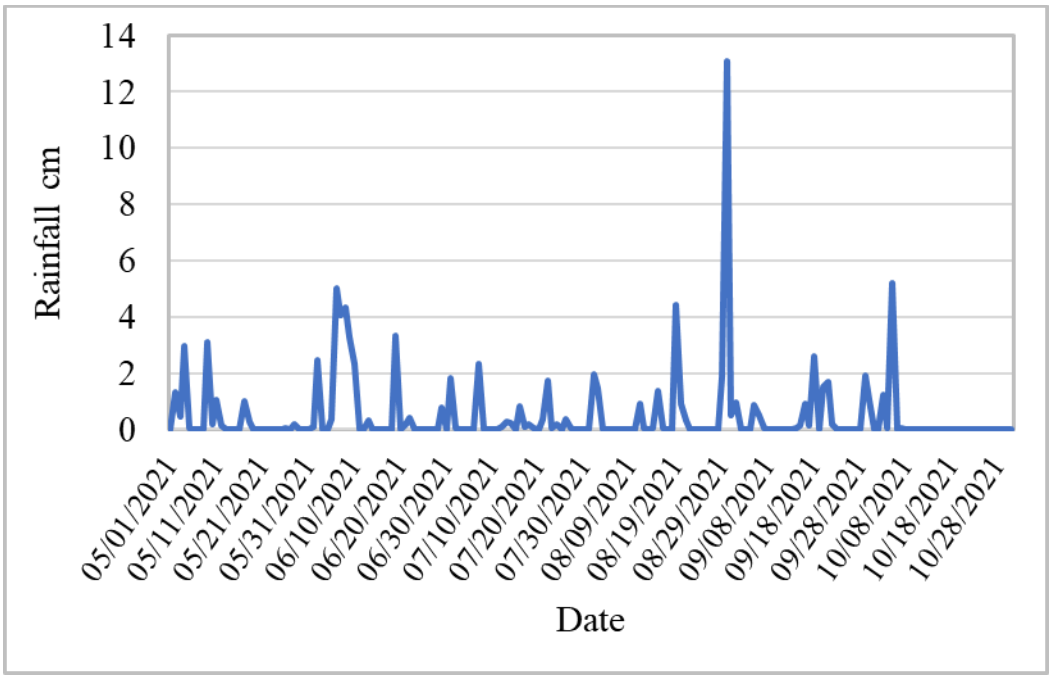

Figure A.5 Daily rainfall in cm from 1 May 2021 to 31 Oct. 2021 recorded at the R.R. Foil Plant Science Research Center in Starkville, MS. Total rainfall was 92.25 cm.

Fresh Weight Tables

Table A.1 ANOVA results from SAS comparing the fresh weight yields of the plant cane years.

R-Square	Coeff Var	Root MSE	Yield Mean
0.779234	26.59759	15.22888	57.25661

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	16382.83	16382.83	70.64	<0.0001
Genotype	20	11224.09	561.2	2.42	0.0044
Location*Genotype	20	7665.36	383.27	1.65	0.0694
Rep	3	5244.02	1748.01	7.54	0.0002
Genotype*Rep	60	8026.35	133.77	0.58	0.9825

ANOVA indicates significant differences between the plant cane years at the HH Leveck Animal Research Center and the Bearden Dairy Research Center in regard to fresh weight yields. There were also significant differences among genotypes.

Table A.2 Comparisons by genotype of mean fresh weight yields between plant cane years at the HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) in Mg ha^{-1} and percentage change.

Genotype	PC Year HHLARC	PC Year BDRC	Percentage Change
AFRI15-1	31.3	52.42	67.6
AFRI15-3	56.7	63.73	12.4
AFRI15-4	30.7	62.50	103.7
AFRI15-5	48.83	46.35	-5.1
AFRI15-6	46.98	73.12	55.7
AFRI15-7	46.5	87.26	87.7
AFRI15-8	59.28	71.38	20.4
AFRI15-9	19.95	82.93	315.7
AFRI15-11	49.05	67.12	36.8
AFRI15-12	54.35	73.33	34.9
AFRI15-13	54.20	55.77	2.9
AFRI15-15	31.47	59.20	88.1
AFRI15-18	46.28	50.67	9.5
AFRI15-19	45.45	65.09	43.2
AFRI15-20	55.03	80.79	46.8
AFRI15-21	56.53	79.04	39.8
AFRI15-22	51.18	68.81	34.5
AFRI15-23	49.35	42.67	-13.5
AFRI15-24	33.93	71.00	109.3
AFRI15-25	61.05	76.02	24.5
Ho02-113	51.55	73.72	43.0
Mean	47.10	67.10	41.9

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC columns are reported in Mg ha^{-1}.

Table A.3 ANOVA results from SAS comparing the fresh weight yields of the plant cane year and ratoon year at the HH Leveck Animal Research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.898725	27.04951	11.04440	40.83031

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	6392.86	6392.86	52.41	<0.0001
Genotype	20	22075.42	1103.77	9.05	<0.0001
Year*Genotype	20	9159.22	457.96	3.75	<0.0001
Rep	3	8811.86	2937.29	24.08	<0.0001
Genotype*Rep	58	17695.93	305.10	2.5	0.0003

ANOVA indicates significant differences between the plant cane year and the ratoon year at the HH Leveck Animal Research Center in regard to fresh weight yields. There were significant differences among genotypes and replications. There were significant interactions between year and genotype as well as genotype and replication.

Table A.4 Comparison by genotype of mean fresh weight yields between plant cane year and ratoon year at the HH Leveck Animal Research Center in Mg ha^{-1} and percentage change.

Genotype	PC Year	Ratoon Year	Percentage Change
AFRI15-1	31.28	2.56	-91.8
AFRI15-3	56.70	79.60	40.4
AFRI15-4	30.67	9.61	-68.7
AFRI15-5	48.83	26.64	-45.4
AFRI15-6	46.97	34.57	-26.4
AFRI15-7	46.52	14.60	-68.6
AFRI15-8	59.29	28.72	-51.6
AFRI15-9	19.94	21.65	8.6
AFRI15-11	49.07	32.51	-33.7
AFRI15-12	54.34	49.93	-8.1
AFRI15-13	54.21	53.68	-1.0
AFRI15-15	31.48	34.47	9.5
AFRI15-18	46.28	54.45	17.7
AFRI15-19	45.43	31.75	-30.1
AFRI15-20	55.00	24.62	-55.2
AFRI15-21	56.53	28.26	-50.0
AFRI15-22	51.19	31.17	-39.1
AFRI15-23	49.35	23.55	-52.3
AFRI15-24	33.94	43.00	26.7
AFRI15-25	61.05	44.97	-26.3
Ho02-113	51.56	41.93	-18.7
Mean	47.30	34.28	-27.5

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC columns are reported in Mg ha^{-1}.

Dry Matter Tables

Table A.5 ANOVA results from SAS comparing the dry matter yields of the PC years in Mg ha^{-1}.

R-Square	Coeff Var	Root MSE	Yield Mean
0.730524	28.83504	3.600266	12.48573

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	326.9614157	326.9614157	25.22	<0.0001
Genotype	20	472.0429233	23.6021462	1.82	0.0392
Location*Genotype	20	361.7242483	18.0862124	1.40	0.1616
Rep	3	354.5331361	118.1777120	9.12	<0.0001
Genotype*Rep	60	520.0919565	8.6681993	0.67	0.9384

ANOVA indicated a significant difference between the plant cane years at the HH Leveck Animal Research Center and the Bearden Dairy Research Center and among genotypes in regard to dry matter yield. There were also significant differences among genotypes.

Table A.6 Comparisons by genotype of mean dry matter yields between plant cane years HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) in Mg ha^{-1} and percentage change.

Genotype	PC year HHLARC	PC year BDRC	Percentage Change
AFRI15-1	7.81	13.74	75.9
AFRI15-3	13.24	13.45	1.6
AFRI15-4	7.05	12.04	70.6
AFRI15-5	12.32	10.93	-11.2
AFRI15-6	10.34	14.01	35.5
AFRI15-7	11.04	16.94	53.4
AFRI15-8	13.06	14.49	11.0
AFRI15-9	3.97	15.95	301.2
AFRI15-11	10.76	12.09	12.4
AFRI15-12	12.72	16.33	28.4
AFRI15-13	14.02	13.40	-4.4
AFRI15-15	7.54	9.65	28.0
AFRI15-18	10.54	9.17	-13.0
AFRI15-19	10.10	13.92	37.7
AFRI15-20	12.73	15.94	25.3
AFRI15-21	12.92	15.05	16.6
AFRI15-22	11.51	14.55	26.4
AFRI15-23	11.98	8.51	-29.0
AFRI15-24	8.36	15.13	80.9
AFRI15-25	15.17	15.20	0.2
Ho02-113	12.34	16.06	30.2
Mean	11.1	13.9	24.9

Genotypes are listed in numerical order with control (Ho 02-113) and mean of all genotypes at the bottom. Yields in PC columns are reported in Mg ha^{-1}.

Table A.7 ANOVA results from SAS comparing the dry matter yields of the plant cane year and ratoon year of the HH Leveck Animal Research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.900654	28.61522	2.934921	10.25650

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	108.420181	108.420181	12.59	0.0008
Genotype	20	1761.22489	88.061245	10.22	<.0001
Year*Genotype	20	782.15946	39.107973	4.54	<.0001
Rep	3	694.232409	231.410803	26.87	<.0001
Genotype*Rep	58	1316.95012	22.706037	2.64	0.0001

ANOVA indicates significant differences between the plant cane year and the ratoon year at the HH Leveck Animal Research Center in regard to dry matter yields. There were significant differences among genotypes. There were significant differences among replications. There were significant interactions between year and genotype as well as genotype and replication.

Table A.8 Comparison by genotype of mean dry matter yields between plant cane year and ratoon year at the HH Leveck Animal research Center in Mg ha^{-1} and percentage change.

Genotype	PC Year	Ratoon Year	Percentage Change
AFRI15-1	7.82	0.68	-91.3
AFRI15-3	13.25	23.66	78.6
AFRI15-4	7.06	2.55	-63.8
AFRI15-5	12.32	8.32	-32.4
AFRI15-6	10.34	8.26	-20.2
AFRI15-7	11.05	3.70	-66.6
AFRI15-8	13.06	6.96	-46.7
AFRI15-9	3.98	4.90	23.1
AFRI15-11	10.77	8.19	-24.0
AFRI15-12	12.72	14.03	10.3
AFRI15-13	14.02	16.23	15.7
AFRI15-15	7.55	9.58	27.0
AFRI15-18	10.55	13.33	26.4
AFRI15-19	10.11	8.72	-13.8
AFRI15-20	12.73	6.243	-51.0
AFRI15-21	12.92	7.17	-44.5
AFRI15-22	11.51	8.52	-26.0
AFRI15-23	11.99	6.32	-47.3
AFRI15-24	8.36	12.41	48.4
AFRI15-25	15.16	12.34	-18.7
Ho02-113	12.34	12.43	0.7
Mean	11.10	9.40	-15.4

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC and ratoon columns are reported in Mg ha^{-1}.

Sap Volume Tables

Table A.9 ANOVA results from SAS comparing the sap volume production of the plant cane years.

R-Square	Coeff Var	Root MSE	Yield Mean
0.925465	7.401369	0.024683	0.333497

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	0.041	0.0415	68.11	<0.0001
Genotype	20	0.266	0.0133	21.9	<0.0001
Location*Genotype	20	0.027	0.0013	2.3	0.0073
Rep	3	0.011	0.0038	6.24	0.001
Genotype*Rep	60	0.054	0.0009	1.5	0.0624

ANOVA indicated a significant difference between the plant cane years at the HH Leveck Animal Research Center and the Bearden Dairy Research Center in regard to sap volume production (ml g^{-1} fresh cane). There were also significant differences among genotypes.

Table A.10 Comparison by genotype of mean sap volume production of plant cane years at the HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) in ml g^{-1} and percentage change.

Genotype	PC year HHLARC	PC year BDRC	Percentage Change
AFRI15-1	0.26	0.34	30.8
AFRI15-3	0.333	0.325	-2.4
AFRI15-4	0.273	0.32	17.2
AFRI15-5	0.258	0.293	13.6
AFRI15-6	0.36	0.38	5.6
AFRI15-7	0.335	0.375	11.9
AFRI15-8	0.393	0.44	12.0
AFRI15-9	0.415	0.438	5.5
AFRI15-11	0.328	0.405	23.5
AFRI15-12	0.275	0.315	14.5
AFRI15-13	0.313	0.3	-4.2
AFRI15-15	0.323	0.338	4.6
AFRI15-18	0.335	0.368	9.9
AFRI15-19	0.328	0.365	11.3
AFRI15-20	0.343	0.378	10.2
AFRI15-21	0.372	0.383	3.0
AFRI15-22	0.29	0.375	29.3
AFRI15-23	0.328	0.353	7.6
AFRI15-24	0.258	0.305	18.2
AFRI15-25	0.315	0.343	8.9
Ho02-113	0.258	0.245	-5.0
Mean	0.3	0.4	10.3

Genotypes are listed in numerical order with control (Ho02-113) and mean at the bottom. SV yields in PC columns are reported in ml g^{-1} of fresh cane.

Table A.11 ANOVA results from SAS comparing the sap volume production of the plant cane year and ratoon year of the HH Leveck Animal Research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.862008	9.360385	0.029264	0.312638

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	0.0018	0.0018	2.15	0.1479
Genotype	20	0.2208	0.0110	12.89	<0.0001
Year*Genotype	20	0.0339	0.0016	1.98	0.022
Rep	3	0.0124	0.0041	4.85	0.0043
Genotype*Rep	58	0.0447	0.0007	0.9	0.6551

ANOVA does not indicate significant differences between the plant cane year and ratoon year at the HH Leveck Animal Research Center in regard to sap volume (ml g^{-1} fresh cane). There were significant differences among genotypes. There are significant differences among replications. There were significant interactions between year and genotype as well as genotype and replication.

Table A.12 Comparison by genotype of mean sap volume production between plant cane year and ratoon year at the HH Leveck Animal Research Center in L ha^{-1}.

Genotype	PC Year	Ratoon Year	Percentage Change
AFRI15-1	8087.4	714.68	-91.2
AFRI15-3	11668.23	24301.58	108.3
AFRI15-4	4750.38	2313.00	-51.3
AFRI15-5	9706.83	7225.73	-25.6
AFRI15-6	12021.88	12978.80	8.0
AFRI15-7	9983.18	5683.58	-43.1
AFRI15-8	16922.25	12935.57	-23.6
AFRI15-9	5421.20	10126.15	86.8
AFRI15-11	8790.08	11198.53	27.4
AFRI15-12	7019.08	9056.08	29.0
AFRI15-13	10017.63	18165.25	81.3
AFRI15-15	5035.60	9959.10	97.8
AFRI15-18	12518.23	19678.25	57.2
AFRI15-19	11078.30	12187.43	10.0
AFRI15-20	14808.48	9871.43	-33.3
AFRI15-21	15789.78	15294.18	-3.1
AFRI15-22	14448.68	10430.45	-27.8
AFRI15-23	12510.15	9910.43	-20.8
AFRI15-24	6036.88	11140.90	84.5
AFRI15-25	10936.25	19029.05	74.0
Ho02-113	4860.05	7251.75	49.2
Mean	10229	11425	11.6

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in pc and ratoon columns are reported in ml g^{-1} of fresh cane.

°Brix Tables

Table A.13 ANOVA results from SAS comparing the °Brix values of the plant cane years.

R-Square	Coeff Var	Root MSE	Yield Mean
0.781272	12.78105	1.547824	12.11030

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	41.04	41.04	17.13	0.0001
Genotype	20	214.62	10.73	4.48	<0.0001
Location*Genotype	20	92.89	4.64	1.94	0.0255
Rep	3	9.53	3.18	1.33	0.2742
Genotype*Rep	60	155.35	2.59	1.08	0.3823

ANOVA indicated a significant difference between the plant cane years at the HH Leveck Animal Research Center and the Bearden Dairy Research Center in regard to °Brix values. There were also significant differences among genotypes. There was an interaction effect between location and genotype.

Table A.14 Comparison by genotype of mean °Brix values of two plant cane years at the HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) and percentage change.

Genotype	PC year HHLARC	PC year BDRC	Percentage Change
AFRI15-1	13.71	15.55	13.4
AFRI15-3	12.79	12.4	-3.0
AFRI15-4	10.00	11.3	13.0
AFRI15-5	11.09	13.7	23.6
AFRI15-6	12.26	13.98	14.0
AFRI15-7	12.56	10.28	-18.2
AFRI15-8	10.75	11.65	8.4
AFRI15-9	11.83	12.70	7.4
AFRI15-11	8.96	11.95	33.3
AFRI15-12	12.64	10.53	-16.7
AFRI15-13	12.66	15.03	18.7
AFRI15-15	11.24	14.48	28.8
AFRI15-18	9.78	10.18	4.1
AFRI15-19	12.20	13.20	8.2
AFRI15-20	9.65	12.05	24.9
AFRI15-21	10.96	12.05	9.9
AFRI15-22	11.68	13.23	13.3
AFRI15-23	12.363	14.4	16.5
AFRI15-24	12.2	12.03	-1.4
AFRI15-25	13.46	12.38	-8.1
Ho02-113	11.11	12.15	9.3
Mean	11.61	12.61	8.6

Genotypes are listed in numerical order with control (Ho02-113) at the bottom and mean of all genotypes at the bottom.

Table A.15 ANOVA results from SAS comparing the sap volume production of the plant cane year and ratoon year of the HH Leveck Animal Research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.860781	11.17569	1.480950	13.25153

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	425.89	425.89	194.19	<0.0001
Genotype	20	185.40	9.27	4.23	<0.0001
Year*Genotype	20	37.97	1.89	0.87	0.628
Rep	3	5.82	1.94	0.88	0.4543
Genotype*Rep	58	133.81	2.30	1.05	0.4227

ANOVA indicated significant differences between the plant cane year and ratoon year at the HH Leveck Animal Research Center in regard to sap °Brix value. There were significant differences among genotypes.

Table A.16 Comparison by genotype of mean °Brix values between plant cane year and Ratoon Year at the HH Leveck Animal research Center.

Genotype	PC Year	Ratoon Year	Percentage Change
AFRI15-1	13.7	16.08	17.2
AFRI15-3	12.79	15.78	23.4
AFRI15-4	10	14.8	48.0
AFRI15-5	11.09	14.48	30.5
AFRI15-6	12.26	16.15	31.7
AFRI15-7	12.56	13.58	8.1
AFRI15-8	10.75	12.9	20.0
AFRI15-9	11.83	14.2	20.1
AFRI15-11	8.96	13.45	50.1
AFRI15-12	12.64	14.68	16.1
AFRI15-13	12.66	16.15	27.5
AFRI15-15	11.24	15.38	36.8
AFRI15-18	9.78	12.78	30.7
AFRI15-19	12.2	14.95	22.5
AFRI15-20	9.65	13.46	39.1
AFRI15-21	10.96	14.56	32.5
AFRI15-22	11.68	16.28	39.4
AFRI15-23	12.36	15.95	29.0
AFRI15-24	12.2	15.13	24.0
AFRI15-25	13.46	15.83	17.5
Ho02-113	11.11	15.9	43.1
Mean	11.6085	14.9148	28.5

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom.

Theoretical Ethanol from Sap Tables

Table A.17 ANOVA results from SAS comparing the theoretical ethanol from sap of the PC years.

R-Square	Coeff Var	Root MSE	Yield Mean
0.861756	39.89893	374.3047	938.1321

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	28025897.4	28025897.4	200.04	<0.0001
Genotype	20	9199052.71	459952.64	3.28	0.0002
Location*Genotype	20	5016820.39	250841.02	1.79	0.0431
Rep	3	472466.93	157488.98	1.12	0.3465
Genotype*Rep	60	6238167.41	103969.46	0.74	0.8746

ANOVA indicated a significant difference between the plant cane years at the HH Leveck Animal Research Center and the Bearden Dairy Research Center in regard to theoretical ethanol from sap. There were also significant differences among genotypes. There was an interaction effect between location and genotype, as well as between genotype and replication.

Table A.18 Comparison by genotype of mean theoretical ethanol from sap between plant cane years at the HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) in L ha^{-1} and percentage change.

Genotype	PC Year HHLARC	PC Year BDRC	Percentage Change
AFRI15-1	471.13	1291.03	174.0
AFRI15-3	674	1178.4	74.8
AFRI15-4	210.45	805.8	282.9
AFRI15-5	461.03	1169.58	153.7
AFRI15-6	654.03	1760.45	169.2
AFRI15-7	553.08	1430.38	158.6
AFRI15-8	791.15	1681.7	112.6
AFRI15-9	247.05	2492.98	909.1
AFRI15-11	322.73	1562.93	384.3
AFRI15-12	396.78	1076.23	171.2
AFRI15-13	547.83	905.93	65.4
AFRI15-15	249.78	1032.9	313.5
AFRI15-18	580.98	888.88	53.0
AFRI15-19	589.45	1571.78	166.7
AFRI15-20	648.58	1774.55	173.6
AFRI15-21	748.05	1915.95	156.1
AFRI15-22	736.93	1502.4	103.9
AFRI15-23	664.75	996.63	49.9
AFRI15-24	331.75	1071.28	222.9
AFRI15-25	617.48	1354.28	119.3
Ho02-113	235.53	874.15	271.1
Mean	517.51	1353.69	161.6

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC columns are reported in Mg ha^{-1}.

Table A.19 ANOVA results from SAS comparing the theoretical ethanol from sap of the plant cane year and ratoon year at the HH Leveck Animal Research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.837858	45.81144	288.3175	629.3571

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	2018722.04	2018722.04	24.28	<0.0001
Genotype	20	9572479.40	478623.97	5.76	<0.0001
Year*Genotype	20	4454637.1	222731.85	2.68	0.0017
Rep	3	1581779.54	527259.85	6.34	0.0008
Genotype*Rep	58	7966749.50	137357.75	1.65	0.0278

ANOVA indicated a significant difference between plant cane year and ratoon year in regard to theoretical ethanol from sap. There were significant differences among genotypes. There were significant differences among replications. There was an interaction effect between year and genotype, as well as, between genotype and replication.

Table A.20 Comparison by genotype of mean theoretical ethanol yield from sap in L ha^{-1} between plant cane year and ratoon year at the HH Leveck Animal Research Center.

Genotype	PC year	Ratoon year	Percentage Change
AFRI15-1	471.13	51.33	-89.1
AFRI15-3	674.00	1649.23	144.7
AFRI15-4	210.45	147.30	-30.0
AFRI15-5	461.03	467.55	1.4
AFRI15-6	654.03	903.83	38.2
AFRI15-7	553.08	332.23	-39.9
AFRI15-8	791.15	736.77	-6.9
AFRI15-9	247.05	626.60	153.6
AFRI15-11	322.73	654.85	102.9
AFRI15-12	396.78	583.68	47.1
AFRI15-13	547.83	1286.48	134.8
AFRI15-15	249.78	676.33	170.8
AFRI15-18	580.98	1109.92	91.0
AFRI15-19	589.45	792.63	34.5
AFRI15-20	648.575	574.48	-11.4
AFRI15-21	748.05	986.02	31.8
AFRI15-22	736.93	752.13	2.1
AFRI15-23	664.75	687.00	3.3
AFRI15-24	331.75	714.6	115.4
AFRI15-25	617.48	1302.5	110.9
Ho02-113	235.53	499.43	112.0
Mean	517.51	742.59	43.5

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC and ratoon columns are reported in L ha^{-1}.

Theoretical Ethanol from Dry Matter Tables

Table A.21 ANOVA results from SAS comparing the theoretical ethanol from dry matter of the plant cane years.

R-Square	Coeff Var	Root MSE	Yield Mean
0.728246	29.54212	638.9628	2162.887

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	9208315.62	9208315.62	22.55	<0.0001
Genotype	20	16751273.1	837563.65	2.05	0.017
Location*Genotype	20	10786869.4	539343.47	1.32	0.2018
Rep	3	12521923.6	4173974.56	10.22	<0.0001
Genotype*Rep	60	16139914.5	268998.58	0.66	0.9456

ANOVA indicated a significant difference between the HH Leveck Animal research Center and the Bearden Dairy Research Center in regard to theoretical ethanol from dry matter. There were significant differences among genotypes. There were also significant differences among replications.

Table A.22 Comparison by genotype of mean theoretical ethanol from sap values of two plant cane years at the HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) in L ha^{-1} and percentage change.

Genotype	PC year HHLARC	PC year BDRC	Percentage Change
AFRI15-1	1361.2	2394.0	75.9
AFRI15-3	2307.1	2343.7	1.6
AFRI15-4	1229.6	2097.6	70.6
AFRI15-5	2146.6	1905.8	-11.2
AFRI15-6	1801.3	2441.4	35.5
AFRI15-7	1924.5	2951.9	53.4
AFRI15-8	2275.6	2525.1	11.0
AFRI15-9	692.8	2779.1	301.2
AFRI15-11	1876.1	2107.8	12.4
AFRI15-12	2215.8	2846.0	28.4
AFRI15-13	2442.7	2335.1	-4.4
AFRI15-15	1314.8	1682.7	28.0
AFRI15-18	1837.5	1598.2	-13.0
AFRI15-19	1761.0	2425.5	37.7
AFRI15-20	2218.0	2778.4	25.3
AFRI15-21	2250.9	2623.4	16.5
AFRI15-22	2005.5	2535.2	26.4
AFRI15-23	2088.4	1482.6	-29.0
AFRI15-24	1456.9	2635.7	80.9
AFRI15-25	2643.8	2649.4	0.2
Ho02-113	2150.0	2798.2	30.1
Mean	1934.3	2388.7	23.5

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC columns are reported in L ha^{-1}.

Table A.23 ANOVA results from SAS comparing the theoretical ethanol from dry matter of the plant cane year and the ratoon year at the HH Leveck Animal research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.900625	28.61771	511.3291	1786.758

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	3289440.4	3289440.4	12.58	0.0008
Genotype	20	53441402.0	2672070.1	10.22	<.0001
Year*Genotype	20	23730789.9	1186539.5	4.54	<.0001
Rep	3	21067010.3	7022336.77	26.86	<.0001
Genotype*Rep	58	39964548.5	689043.94	2.64	0.0001

ANOVA indicated a significant difference between plant cane year and ratoon year in regard to theoretical ethanol from dry matter. There were significant differences among genotypes. There were significant differences among replications. There was an interaction effect between year and genotype, as well as, between genotype and replication.

Table A.24 Comparison by genotype of mean theoretical ethanol yield from dry matter in L ha^{-1} between plant cane year and ratoon year at the HH Leveck Animal Research Center.

Genotype	PC Year	Ratoon Year	Percentage Change
AFRI15-1	1361.2	119.2	-91.2
AFRI15-3	2307.1	4121.4	78.6
AFRI15-4	1229.6	445.2	-63.8
AFRI15-5	2146.6	1449.5	-32.5
AFRI15-6	1801.3	1438.7	-20.1
AFRI15-7	1924.5	643.8	-66.5
AFRI15-8	2275.6	1212.7	-46.7
AFRI15-9	692.8	852.4	23.0
AFRI15-11	1876.1	1425.7	-24.0
AFRI15-12	2215.8	2443.7	10.3
AFRI15-13	2442.7	2827.2	15.7
AFRI15-15	1314.8	1668.7	26.9
AFRI15-18	1837.5	2322.2	26.4
AFRI15-19	1761.0	1518.2	-13.8
AFRI15-20	2218.0	1087.2	-51.0
AFRI15-21	2250.9	1248.8	-44.5
AFRI15-22	2005.5	1484.4	-26.0
AFRI15-23	2088.4	1101.3	-47.3
AFRI15-24	1456.9	2161.9	48.4
AFRI15-25	2643.8	2149.7	-18.7
Ho02-113	2150.0	2164.7	0.7
Mean	1934.3	1637.4	-15.4

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC and ratoon columns are reported in L ha^{-1}.

Total Theoretical Ethanol Tables

Table A.25 ANOVA results from SAS comparing the total theoretical ethanol yield of the plant cane years.

R-Square	Coeff Var	Root MSE	Yield Mean
0.796300	29.08301	907.8833	3121.696

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Location	1	73680933.3	73680933.3	89.39	<0.0001
Genotype	20	36418059.4	1820902.97	2.21	0.0096
Location*Genotype	20	27233829.5	1361691.48	1.65	0.0695
Rep	3	22002750.7	7334250.24	8.9	<0.0001
Genotype*Rep	60	29287749.8	488129.16	0.59	0.9777

ANOVA indicated a significant difference between the plant cane years at the HH Leveck Animal Research Center in regard to total theoretical ethanol. There were also significant differences among genotypes. There were significant differences among replications.

Table A.26 Comparison by genotype of mean total theoretical ethanol yield of two plant cane years at the HH Leveck Animal Research Center (HHLARC) and the Bearden Dairy Research Center (BDRC) in L ha^{-1} and percentage change.

Genotype	PC year HHLARC	PC year BDRC	Percentage Change
AFRI15-1	1832.3	3685.0	101.1
AFRI15-3	2981.1	3522.1	18.1
AFRI15-4	1440.1	2903.4	101.6
AFRI15-5	2607.6	3075.3	17.9
AFRI15-6	2455.3	4201.9	71.1
AFRI15-7	2477.6	4382.2	76.9
AFRI15-8	3066.7	4206.8	37.2
AFRI15-9	939.9	5272.1	460.9
AFRI15-11	2198.7	3670.7	66.9
AFRI15-12	2612.6	3679.4	40.8
AFRI15-13	2990.6	3262.2	9.1
AFRI15-15	1564.5	2715.6	73.6
AFRI15-18	2418.5	2487.2	2.8
AFRI15-19	2350.4	3997.3	70.1
AFRI15-20	2866.5	4553.0	58.8
AFRI15-21	2998.9	4539.3	51.4
AFRI15-22	2742.4	4037.5	47.2
AFRI15-23	2753.2	2479.2	-10.0
AFRI15-24	1788.6	3707.0	107.3
AFRI15-25	3261.2	4003.6	22.8
Ho02-113	2385.5	4746.9	99.0
Mean	2451.8	3783.5	54.3

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. PC columns are reported in L ha^{-1}.

Table A.27 ANOVA results from SAS comparing the total theoretical ethanol yield of the plant cane year and ratoon year at the HH Leveck Animal Research Center.

R-Square	Coeff Var	Root MSE	Yield Mean
0.890082	29.74439	718.6561	2416.107

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year	1	154373.53	154373.53	0.3	0.5866
Genotype	20	96916690.4	4845834.52	9.38	<.0001
Year*Genotype	20	45223956.2	2261197.81	4.38	<.0001
Rep	3	33973792.3	11324597.46	21.93	<.0001
Genotype*Rep	58	73689683.6	1270511.79	2.46	0.0003

ANOVA indicated a significant difference between plant cane year and ratoon year in regard to total theoretical ethanol yield. There were significant differences among genotypes. There were significant differences among replications. There was an interaction effect between year and genotype, as well as, between genotype and replication.

Table A.28 Comparison by genotype of mean total theoretical ethanol yield in L ha^{-1} between PC year and ratoon year at the HH Leveck Animal Research Center.

Genotype	PC Year	Ratoon Year	Percentage Change
AFRI15-1	1832.3	170.5	-90.7
AFRI15-3	2981.1	5770.6	93.6
AFRI15-4	1440.1	592.6	-58.9
AFRI15-5	2607.6	1917.1	-26.5
AFRI15-6	2455.3	2342.5	-4.6
AFRI15-7	2477.6	976.0	-60.6
AFRI15-8	3066.7	1949.5	-36.4
AFRI15-9	939.9	1479.0	57.4
AFRI15-11	2198.7	2080.5	-5.4
AFRI15-12	2612.6	3027.4	15.9
AFRI15-13	2990.6	4113.7	37.6
AFRI15-15	1564.5	2345.0	49.9
AFRI15-18	2418.5	3432.1	41.9
AFRI15-19	2350.4	2310.8	-1.7
AFRI15-20	2866.5	1661.7	-42.0
AFRI15-21	2998.9	2234.7	-25.5
AFRI15-22	2742.4	2236.5	-18.4
AFRI15-23	2753.2	1788.2	-35.0
AFRI15-24	1788.6	2876.5	60.8
AFRI15-25	3261.2	3452.3	5.9
Ho02-113	2385.5	2664.1	11.7
Mean	2451.8	2380.0	-2.9

Genotypes are listed in numerical order with control (Ho02-113) and mean of all genotypes at the bottom. Yields in PC and ratoon columns are reported in L ha^{-1}.