SUGAR, BIOMASS AND BIOFUEL POTENTIAL OF
TEMPERATE BY TROPICAL MAIZE CROSSES

BY

WENDY GAIL WHITE

THESIS

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Adviser:

Professor Frederick E. Below

ABSTRACT

In order to shift society's dependence away from petroleum the U.S. Department of Energy has set goals to replace 30% of petroleum based fuels with biofuel by the year 2030. This has produced an unprecedented interest in producing biofuels such as ethanol from plant biomass. A wide variety of potential biomass sources are currently under consideration, including grass species that employ the C4 pathway for photosynthesis, which include the perennial grasses, miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum* L.), maize (*Zea mays* L.), sugarcane (*Saccharum* spp.), and sorghum (*Sorghum bicolor* L. Moench). These plants exhibit the highest potential for biofuel production because, depending upon the species, they can accumulate sugar, starch and/or lignocellulosic biomass. The perennial grasses are being considered for lignocellulosic fermentation, but this biofuel conversion process is not yet commercially viable. Current ethanol supplies are primarily produced via direct fermentation from the grain of maize in the U.S. and from sugarcane in Brazil. Similar to sugarcane, maize has the ability to store soluable sugars in the stalk, particularly when grain formation is impeded, either through mechanical ear removal, male sterility, or reproductive asynchrony. When tropically-adapted photoperiod sensitive maize varieties are grown in temperate climates, they produce tall plants that produce little to no grain because pollen sheds from the tassel before the silks emerge from the ears. As a result photoassimilates are retained in the stalk as sugar. The growth and use of maize for stalk sugar is believed to have been the impetus for its domestication, and has been reportedly used for this

purpose by the Aztecs and colonial Americans, and has been the subject of modern academic evaluations throughout the current and last century. Like many traits in maize, genotypic variation for stalk sugar, as well as other desirable biofuel traits (i.e. biomass, nitrogen use efficiency, and drought tolerance) is clearly present in both temperate and tropical materials, which indicates the potential for improvement from genetic selection. When temperate and tropical maize are crossed, the progeny plants are better adapted to a temperate climate and are still tall, but exhibit minimal lodging, produce little grain and have the potential to accumulate large amounts of sugar in the stalk, similar to sugarcane. These traits indicate that the temperate x tropical maize (TTM) hybrids may potentially have high value as both as a sugar and lignocellulosic biofuel feedstock.

In order to better determine the utility of TTM as a dedicated biofuel crop, we evaluated a series of TTM hybrids in 2008 and 2009 for their biological potential, genetic variability, the impact of nitrogen (N) on biomass and stalk sugar, and the subsequent biofuel potential. The TTM hybrids produced on average nearly 11 U.S. tons of biomass/acre, and when grain formation was prevented by ear shoot-bagging. TTM produced over 4,360 lbs/acre of sugar, which was three to four-fold greater than the non-ear shoot-bagged TTM and the ear removed hybrids. Taking into consideration the ethanol potential from sugar, stover, and grain, calculated estimates for ethanol production indicate TTM hybrids can yield the same amount of ethanol per acre as modern grain hybrids but with a lower requirement for supplemental fertilizer N. Temperate x tropical maize hybrids developed and selected for desirable biofuel attributes have the potential to be a sustainable biorefinery feedstock.

This thesis is formatted in two chapters; the first provides a literature review pertaining to maize as a sugar and lignocellulosic biofuel feedstock. Elements of this were included in a book chapter review. The second chapter is a manuscript drafted in accordance with the guidelines set forth by the publication Global Change Biology Bioenergy, Wiley – Blackwell publishers.

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TABLE OF CONTENTS

CHAPTER 1

A REVIEW OF THE LITERATURE PERTAINING TO SUGAR AND LIGNOCELLULOSIC BIOFUEL POTENTIAL OF MAIZE

1.1 BIOFUEL FEEDSTOCKS

The United States is the largest user of energy in the world with the U.S. Energy Information Administration reporting that the United States accounted for about 21% of total worldwide energy consumption in 2006 (US DOE, 2009). Change in global energy use will require a change in U.S. production and consumption of energy. Issues of global climate change, national energy security, and boosting rural economies provide incentives to produce ethanol and other fuels from bio-based feedstocks that include cellulosic plant materials. Converting a portion of the world's fuel supply from petroleum based products will require an inexpensive, uniform, and renewable source of biomass, of which agricultural sources are expected to provide a substantial amount (Perlack *et al*., 2005). However, no single feedstock type or land management practice will work for all locations. A joint 2009 workshop report from the U.S. Department of Agriculture (USDA) and Department of Energy (DOE) concluded that it is necessary to view the whole system from production, management, and processing to economic outputs, infrastructure and resource requirements for different feedstocks (BRDI, 2009). The U.S. has access to significant amounts of biobased resources including those of the highly productive corn-soybean cropping system. This agro-ecosystem is still largely focused on providing raw materials for the food and feed industries rather than the biofuel industry.

The increasing demand for biofuels and the desire to avoid drawing that biofuel from the food supply has created a market for new biomass crops as feedstock to support the growing bioenergy industry (USDA DOE, 2008).

Determining the traits or characteristics needed for "a good biomass crop" can be a challenge due to the diversity of environments and the varieties of feedstocks under consideration. Further complications include economic factors related to costs of planting, harvest and transportation, yield per acre, fuel conversion efficiency (fuel yield), infrastructure for all parts of the crop life cycle, and potential barriers if growers are reluctant to take the risk of growing a "new crop" (White *et al.* 2010). Desirable attributes associated with biofuel crops include but, are not limited to, genetic resources for improvement, lack of invasiveness, latitudinal range of adaptability, availability of production and harvest equipment, and grower acceptance. (BRDI, 2009)

A wide variety of plant species have been proposed as potential biomass crops to meet this need, each offering complementary advantages in different regions based on a number of important biological, economic, environmental, and societal factors. Among the many possible options for biomass crops, grass species that employ the C4 pathway for photosynthesis emerge as leading candidates because they exhibit the highest efficiencies of carbon fixation, water use, and nitrogen economy (Ragauskas *et al.*, 2006).

Maize (*Zea mays* L.), sugarcane (*Saccharum officinarum*), sorghum (*Sorghum bicolor* L. Moench) and switchgrass (*Panicum virgatum* L.) all belong to this group of C4 grasses and are already highly productive biomass crops that collectively are cultivated across the global spectrum of agricultural production environments (US DOE, 2006). Depending on the species, C4 grasses can produce one or more of three distinct types of

biomass feedstocks: sugar, starch, and lignocellulosic biomass. Although different C4 grass crops may be specialized for one or more of these forms of harvested carbon, most also exhibit versatility in the types of carbon produced and thus can be tailored as appropriate feedstocks for specific environments or processing streams (Vermerris *et al.*, 2007).

Corn grain is one of the primary sources for ethanol in the U.S. and Brazil has been producing ethanol from sugarcane juice for over thirty years (Andrietta *et al.*, 2007). Corn stover (residue remaining on the field after grain harvest), sorghum, sugarcane, miscanthus (*Miscanthus x giganteus*) and switchgrass are some of the major biomass feedstocks in the U.S. considered for lignocellulosic biofuel production including bioethanol and biobutanol (BRDI 2009). Sugarcane and sweet sorghum are unique among these feedstocks because they exhibit the desirable combination of readily fermentable sugar in addition to lignocellulosic biomass (US DOE, 2006).

Brazil successfully produces 4.2 billion gallons of ethanol per year by directly fermenting the plant sugars from sugarcane to biofuel (Yuan *et al.*, 2008). The sugar extracted from the stalk is used to produce not only ethanol but also economically high value food grade sugar, molasses, and other byproducts, with the remaining lignocellulosic component (bagasse) thermally converted into steam and electricity to power the sugar biorefinery or it is sold to the power grid (Pandey *et al.*, 2000, Yuan *et al.*, 2008). However, due to its adaptation to warm temperate to tropical areas the production of sugarcane is limited to the southernmost states in the U.S. and may only fulfill a small geographical niche in this country (Shapouri *et al.*, 2006).

Sorghum (*Sorghum biocolor* L. Moench), a dual purpose lignocellulosic/sugar feedstock is receiving considerable interest for its versatility in carbon forms and is a potential temperate alternative to sugarcane and an adjunct feedstock to corn grain-based ethanol production (Rooney *et al.*, 2007, Sarath *et al.*, 2008). It is suggested that high biomass sorghums exhibit promise as a dedicated bioenergy crop due to their high yield potential (nearly 10 U.S. tons dry matter/acre) and as some studies have indicated, over 20% of the biomass is sugar based on dry weight (Rooney *et al.*, 2007). Sorghum has a wide range of adaptation, requires low fertilizer inputs, exhibits drought tolerance, and has a well established production system. Theoretical lignocellulosic ethanol yields for sweet sorghum including the yield potential of both biomass and extracted juice are comparable to yields of ethanol produced from corn grain, but requiring lower fertilizer inputs (Propheter *et al.*, 2010).

Unlike ethanol produced from corn grain, which has an energy balance ratio of 1.4 to 1.5 (i.e. energy invested versus energy returned), the combined sugar and biomass of sorghum and sugarcane would have an energy balance ratio approaching 10 (Goldemberg, 2007). Therefore, these crops can provide more energy per unit of land area than corn grain ethanol and, as a result, are more efficient in land usage. Sorghum and sugarcane have the potential to reduce greenhouse gas emissions by combining two of the most effective feedstocks, sugar and lignocellulosic biomass. In comparison to gasoline, ethanol made from cellulose and produced with power generated from burning biomass byproducts can result in an 86 percent reduction in greenhouse gas emissions (Wang *et al*., 2007). This release of greenhouse gas can be reduced still further if the CO_2

released from fermentation is recaptured photosynthetically into additional feedstock (Wang *et al*., 2007).

1.2 MAIZE AS A STARCH, LIGNOCELLULOSIC AND SUGAR FEEDSTOCK

The U.S. Department of Energy has set goals to replace 30% of petroleum based fuels with biofuel by the year 2030 (BRDI 2009) and more recently the U.S. Environmental Protection Agency (EPA) released its final rule for the expanded Renewable Fuels Standard (RFS2) on February 3, 2010. The RFS2 sets forth production volume standards to be met by 2022 of 36 billion gallons of total renewable biofuels, of which 15 billion gallons are to be ethanol derived from corn starch (from maize grain) and other currently produced biofuels, and an additional 21 billion gallons are to be advanced biofuels to include cellulosic ethanol, biomass based diesel and other biofuels that are not corn starch based (RFA 2010). The mature U.S. corn (maize) ethanol industry, in 2009 produced 10.6 billion gallons of ethanol (RFA 2010). In order to meet these goals for 2022, expansion of the corn ethanol industry is anticipated. Issues that have arisen concerning the use of maize grain for ethanol include the potential for a reduced supply available for other markets including livestock and exports (Propheter *et al.*, 2010); and the fact that maize requires an annual application of high levels of nitrogenous fertilizers for maximum yields, which increases the energy balance for using maize grain as a biofuel feedstock, and can negatively impact water and air quality (Tilman *et al.*, 2006)

In order to meet the RFS2 goals for cellulosic ethanol numerous potential feedstocks are under consideration. One option is to utilize agricultural residues from

current established crop production systems. Corn (maize) stover is the resultant agricultural waste or residue from maize grain production and is under investigation as a potential biomass feedstock (Perlack *et al.*, 2005). However, recent concerns have arisen pertaining to the negative impact to soil organic carbon, quality and productivity that can result from the prolonged removal of corn stover from the land (Blanco-Canqui H & Lal R, 2009).

Although not a new concept, recent interest in sugar rich crops, such as sorghum and sugarcane for energy production have revived interest in the ability of maize to accumulate elevated levels of sugar as sucrose in the stalk (Van Reen & Singleton, 1952). Studies using a variety of cultivars selected for maize grain production indicate that although there is great variability between the cultivars, some cultivars yielded about the same amount of alcohol per acre as sweet sorghum (D'Ayala Valva & De Oliveira, 1980). It was also found that the absence of grain production enhances sugar content in the stalks, and this barrenness can be achieved through high population, male sterility or delayed senescence (Bertolini *et al.*, 1993). Since there is a high level of genetic variability within the species of maize, it is anticipated that through selection cultivars can be produced with a two to three fold increase in stalk sugar content, resulting in maize cultivars that could yield more alcohol than sweet sorghum and about the same as sugarcane per unit land area (D'Ayala Valva & De Oliveira, 1980).

1.3 BIOLOGICAL PROPERTIES OF MAIZE INFLUENCING STALK SUGARS

Maize (*Zea mays* L.) is a remarkable crop species adapted from its tropical origins to a wide diversity of environments and economic products. According to the Food and

Agriculture Organization of the United Nations, 873 million U.S. tons of maize was produced world-wide in 2007, making it the world's highest yielding grain crop (FAOSTAT 2009).

When maize varieties adapted to tropical latitudes are grown in temperate environments such as the U.S. Corn Belt, they flower later and produce little or no grain, but have higher total biomass yields compared to modern commercial corn grain hybrids (Stevenson JC & Goodman, 1972). Although offering potential benefits as a feedstock for biofuels, the direct use of tropical maize germplasm in temperate environments is hampered by greater lodging, less stress tolerance, and susceptibility to disease and insect pests; traits that have been greatly improved in modern U.S. corn grain hybrids (Holland & Goodman, 1985).

Maize is classified as a "short-day" plant, meaning that the duration of the night must be longer than a critical threshold to trigger flowering. As a species that evolved in the tropics, maize flowers under approximately 12 hour day/night cycles (Mungoma & Pollak, 1991). Altering this photoperiod control to trigger flowering during the longer days and shorter nights of summer seasons in higher latitudes was a key factor in adaptation of maize to temperate environments like the U.S. Corn Belt. Growing maize varieties adapted to the tropics in temperate environments results in several developmental changes associated with sensitivity to photoperiod. The rate of shoot maturation is slower, leading to more vegetative leaves, thicker stalks, and delayed flowering with disrupted coordination between production of pollen from the male tassel and emergence of the silks on female ears (King *et al.*, 1972) and also accumulates greater amounts of extractable stalk sugar (sucrose, glucose and fructose) because of

7

reduced grain formation (Van Reen & Singleton, 1952). Any seeds that are fertilized

develop more slowly (King *et al.*, 1972). Each of these environmental responses provides

the benefit of prolonged photosynthesis and carbon fixation. When coupled with

reductions in grain formation, the sugar from photosynthesis is not translocated to grain

and converted to starch, but is instead retained in the stalk as sugar (Van Reen &

Singleton, 1952).

1.4 THE HISTORY OF MAIZE AS A SUGAR CROP.

      The concept of exploiting maize for its potential sugar and ethanol production has

a long history. Anthropologists suggest a primary driver for the domestication of maize

was the high sugar content from stalks of its wild ancestor teosinte (*Zea* spp.), which may

have had many uses including fermentation to alcohol for human consumption (Smalley

& Blake, 2003). The Aztecs made sugar from maize stalks long before the European

discovery of the New World (Winton & Winton, 1939). Throughout the past century,

there are periodic reports evaluating maize as a potential sugar crop for alternative

sources of table sugar (Clark, 1913), alcohol production (Widstrom *et al.*, 1984), or a

higher value animal feed (Sayre *et al*. 1931; Singleton, 1948; Campbell, 1964; Marten &

Westerberg 1972; Stake *et al*., 1973; Leshem & Wermke, 1981). Each of these

investigations showed that sugar concentrations and yields increased when grain

production is minimized, either by severe drought stress, high plant population density,

prevention of pollination by covering ears, physical removal of the ear following

pollination or genetic male sterility. However, these studies only considered temperate-

adapted germplasm, and only one study included a tropical line among the temperate-adapted varieties (King *et al.*, 1972).

The stalk serves as a conduit for movement of sugars to developing seeds, and evaluations of maize as a sugar crop demonstrate that the stalk is also an alternate sink for sugars when photoassimilate production exceeds plant use (Crafts-Brandner *et al.*, 1984). In addition to the rapid accumulation of sugars in the stalk, the link between assimilate production and utilization results in accelerated leaf senescence following removal of the earshoot or the prevention of seed formation in corn grain hybrids (Crafts-Brandner *et al.*, 1984). The limited production of grain from tropical hybrids grown in temperate regions does not cause this accelerated leaf senescence, conversely, the plants remain green longer and retain sugars in their stalks (Bertolini *et al.*, 1993). A combination of sucrose, glucose and fructose begins to accumulate around the time of silk emergence but the majority of stalk sugar is in the form of sucrose (Van Reen & Singleton, 1952).

1.5 GENETIC POTENTIAL FOR INCREASING STALK SUGAR IN MAIZE

Maize is an outstanding genetic model species with the best potential for tailoring biomass for its end use (Carpita & McCann, 2008). The tremendous, inherent, genetic diversity of all maize can be mined for genes impacting downstream biological, thermal or catalytic conversion for biofuel (Yu *et al.*, 2008). At present, only a handful of genes have been identified in grass species that contribute to biomass quantity or chemical composition (Carpita & McCann, 2008). However, several thousand genes may affect these complex, quantitative, traits, controlling the ratio of primary to secondary-walled cells, cellulose crystallinity, cell wall composition and architecture, plant anatomy or

rates of cell growth (Vermerris *et al*., 2007). The tools of plant molecular biology and genetics enable the production of plants in which these characteristics are optimized.

Biotechnology has also been a valuable means of improving maize and more biotechnology traits have been commercialized for maize than any other crop species (Vermerris *et al*., 2007). Through a combination of breeding and biotechnology approaches, genes can be identified and modified to enhance biomass and sugar production in maize or provide benefits to the downstream saccharification and fermentation processes or other conversion routes (Vermerris *et al*., 2007). Biotechnology traits have yet to be commercially deployed in sorghum, partly due to concerns about gene flow from cultivated sorghum to its close relative johnsongrass (*Sorghum halpense*), a pernicious weed (Morrell *et al*., 2005; Snow *et al*., 2005). In addition, the U.S. biotechnology regulatory framework for dedicated perennial grass crops such as switchgrass and miscanthus is still being defined (Carpita & McCann, 2008).

Due to the diversity and complexity of the burgeoning biomass industry, there is no panacea for our future energy requirements. Instead, a combination of existing mature biofuel technologies, including corn grain, sugarcane ethanol and soy based biodiesel, along with second generation ethanol feedstocks, such as perennial grasses and agricultural residues, and third generation feedstocks, such as algae, requiring research and development to commercialize, will need to be used synergistically to formulate a sustainable energy future.

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CHAPTER 2

THE SUGAR, BIOMASS AND BIOFUEL POTENTIAL OF TEMPERATE BY TROPICAL MAIZE HYBRIDS

2.1 ABSTRACT

The increasing demand for domestic biofuels has created a market for new feedstocks to meet future energy demands. Temperate x tropical maize (*Zea mays L*) hybrids, with the combined attributes of high biomass and readily fermentable stalk sugars, have yet to be considered as a biomass feedstock. The objective of this study was to evaluate the biological potential, genetic variability and the impact of nitrogen (N) on biomass, stalk sugar, and the subsequent biofuel potential of temperate x tropical maize (TTM) hybrids. Twelve TTM hybrids (divided into early and late maturities), two commercial grain and two silage hybrids were grown in 2008, followed in 2009 by seven ear shoot-bagged TTM hybrids. In both years they were grown without and with supplemental N (180 lbs/acre) in Champaign, IL. Plants were sampled for total and partitioned biomass, and the stalk analyzed for concentration and content of stalk sugar. The TTM hybrids were on average 40% taller, exhibited later reproductive maturity, had greater flowering asynchrony, and remained green longer than the other hybrids. All hybrids responded to supplemental N by producing a greater amount of biomass and grain, a lower percent of biomass partitioned to stalk and leaf, while TTM also had a decreased concentration of sugar. Total biomass yields maximized at 10.5 U.S. dry tons/acre were similar between the TTM and grain hybrid. However, TTM partitioned 50% more biomass to the stalk and produced 50% more sugar, and had less than half the grain of the commercial hybrids, indicating grain production and sugar accumulation are

inversely related. When grain formation was prevented by ear shoot-bagging, TTM

hybrids produced, without supplemental N fertilizer, an average of 4,360 lbs/acre of

sugar, which was three to four-fold greater than the non ear shoot-bagged TTM and the

ear removed hybrid. Calculated estimates for ethanol production, considering the

potential from sugar, stover and grain, indicate TTM can yield the same of amount of

ethanol per acre as modern grain hybrids but with a decreased requirement for

supplemental fertilizer N.

2.2 INTRODUCTION

Converting 30% of the U.S. liquid fuel supply from petroleum to biofuels by 2030

will require nearly 1.4 billion tons of inexpensive, uniform and renewable cellulosic

biomass annually, of which one billion tons will come from agricultural resources (Perlak

et al., 2005). Although a wide variety of plant species have been proposed as potential

biomass crops, grass species that employ the C4 pathway for photosynthesis are the

leading candidates because they exhibit the greatest efficiencies of carbon fixation, water

use and nitrogen (N) economy (Ragauskas *et al.*, 2006). The perennial grasses,

miscanthus (*Miscanthus x giganteus*) and switchgrass (*Panicum virgatum*), sugarcane

(S*accharum officinarum*), sorghum (*Sorghum bicolor* L. Moench) and maize (*Zea mays*

L.), all belong to this group of C4 grasses (Yuan *et al.*, 2008).

Perennial grasses have received considerable attention as a biofuel feedstock due

to their ability to recycle much of their plant N which makes them more efficient at

producing dry matter per unit of N and decreases their requirement for supplemental

fertilizer N. However, converting the lignocellulosic components of perennial grasses

into ethanol is currently not commercially feasible, and there is limited knowledge of the genetic resources needed to select for improved cultivars (Carpita & McCann, 2008). As a result, the mature U.S. biofuel industry produces ethanol from maize grain, for which there is extensive genetic knowledge and genomic tools and for which agricultural production practices are well established. However, maize requires an annual application of high levels of nitrogenous fertilizers for maximum yields, which increases the energy balance for using grain as a biofuel feedstock, and can negatively impact water and air quality (Tilman *et al.*, 2006). In contrast to maize grain, Brazil has successfully utilized sugarcane for nearly thirty years to produce ethanol from the readily fermentable sugars extracted from the stalk, while burning the resultant stover or bagasse to generate electricity (Andrietta *et al*., 2007). Similar to maize, sugarcane requires high levels of supplemental N for maximum production (Robertson *et al.*, 1996), but unlike maize can only be produced in the southernmost states in the U.S. (Shapouri *et al*., 2006). Sorghum holds promise as a sugar crop in the U.S. (Rooney *et al.*, 2007) since it is drought tolerant with a fairly low N requirement and can be grown over a larger geographic area than sugarcane, but not as extensive an area as maize.

Although currently grown for grain, maize throughout history has been valued for its ability to accumulate soluable sugars in the stalk, similar to sugarcane and sorghum. Anthropologists suggest a primary driver for the domestication of maize was the elevated sugar content from stalks of its wild ancestor teosinte (*Zea* spp.), that provided humans with a sweet sugar food source, and which could also be fermented into an alcoholic beverage (Smalley & Blake, 2003). Throughout the past century, there are periodic reports evaluating maize as a potential sugar crop for alternative sources of table sugar

(Clark, 1913), alcohol production (Widstrom *et al.*, 1984), or a higher value animal feed (Sayre *et al.*, 1931; Singleton, 1948; Campbell, 1964; Marten & Westerberg, 1972; Stake & Owens *et al.*, 1973; Leshem & Wermke, 1981). Each of these investigations showed that sugar concentration and yield increased when grain production was minimized by means of severe drought stress, high plant population density, prevention of pollination, or by ear removal. The association between assimilate production and utilization is evidenced by the rapid accumulation of sugars in the stalk, and the accelerated leaf senescence that occurs following elimination of the reproductive phase in most commercial maize hybrids (Crafts-Brandner *et al.*, 1984). This accelerated senescence limits the potential for biomass and sugar accumulation by decreasing photosynthesis and assimilate production in the commercial maize hybrids. Conversely, there are few reports of using tropical maize germplasm as a potential sugar source, despite some advantages associated with its sensitivity to photoperiod and its prolonged photosynthesis and assimilate production which may enhance plant sugar production.

Maize varieties adapted to the tropics and grown in a temperate environment exhibit several changes associated with sensitivity to photoperiod, including a slower rate of shoot maturation, more vegetative leaves, thicker stalks, (Stevenson & Goodman, 1972), and delayed flowering with disrupted coordination between production of pollen from the male tassel and emergence of the silks on the female ears (King *et al.* 1972). Each of these physiological responses to the photoperiod provides the benefit of prolonged photosynthesis and carbon fixation. When coupled with reductions in grain formation, the sugar from photosynthesis is not translocated to grain and converted to starch, but is instead retained in the stalk as sugar (Van Reen & Singleton, 1952).

Some disadvantages of tropical maize cultivars, however, are that they exhibit weak stalks and roots and greater disease susceptibility when grown in a temperate climate (Holland & Goodman, 1995). By crossing temperate and tropical parental germplasm, the temperate parent imparts improved agronomic traits such as better disease and pest resistance, decreased lodging, and abiotic stress tolerance (Nelson & Goodman, 2008). Genetic variability resulting from these crosses also extends to the sugar concentration in the stalks and through inbred selection for hybrids, sugar yields can be increased (Widstrom *et al.*, 1984).

Our objective was to investigate the biofuel feedstock potential of hybrids produced by crossing inbred lines of temperate x tropical maize (TTM). A selection of TTM hybrids produced from readily available germplasm for biological potential and genetic variability was grown in 2008 and 2009. The biomass partitioning of TTM was compared to commercial grain corn hybrids with and without ears, and commercial silage hybrids to identify and contrast the physiological aspects of assimilate translocation and storage. In order to better determine stalk sugar potential the 2009 TTM hybrids were ear shoot-bagged to prevent grain formation. The plants were grown with no supplemental nitrogen and with a standard rate of 180 lbs/acre nitrogen to help us further understand the relationship between nitrogen supply, biomass and sugar production. Utilizing the biomass and sugar data we then estimated biofuel potential of the TTM hybrids as compared to commercial grain hybrids.

2.3 MATERIALS AND METHODS

2.3.1 Field site, Cultural Practices and Treatment Arrangements

Field experiments were conducted at the University of Illinois Department of Crop Sciences Research and Education Center in Champaign, Illinois during 2008 and 2009. The soil type was a Flanagan/Drummer silty clay loam with an average organic matter of 4.8% and a pH of 6.1 and a slope less than 5%. The field was under a maize-soybean rotation, with the location of the experimental plots alternated each year. Plots were kept weed-free with using a pre-emergent application of Lumax® (S-Metolachlor + Atrazine + Mesotrione) at a rate of 3 quarts/acre, and hand cultivation. Seeds were treated with Captan (N-trichtoromethylthio-cyclohexene-1.2-dicarboximide) in 2008, and Cruiser® (thiamethoxam) in 2009, and the insecticide Aztec® (tebupirimfos and cyfluthrin) was applied at 7.3 pounds per acre at planting for corn rootworm and other early insect control. Planting occurred on May 29, 2008, and May 27, 2009. Treatments were arranged in a randomized complete block design as a restricted split plot with each hybrid having adjacent nitrogen (N) treatments (0 and 180 lbs supplemental N per acre) and with three replications. Each plot consisted of six 17.5 foot rows, with all rows receiving the N treatment, and with the center three rows used for sampling. The plots were overplanted and thinned to achieve a final stand density of 36,000 plants per acre. Nitrogen treatments were applied as granular ammonium sulfate in a diffuse band in the center of the row at the 3 to 5 leaf stage, (June 18 and June 17 in 2008 and 2009) followed by incorporation with a row cultivator.

2.3.2 Germplasm Selection, Sampling and Measurement, 2008

Hybrids of elite temperate, tropical, and tropical-adapted inbreds of available genetic mapping populations (Yu *et al.*, 2008), different temperate heterotic groups (Mikel, 2006), and a temperate hybrid U.S. tester, LH132 x LH51 (Nelson & Goodman, 2008) were used in this study. These selections were based on the phenotypic trait of substantive biomass (Maize Molecular and Functional Diversity Project, http://www.panzea.org), which is believed to be associated with considerable sugar yields (Bertolini *et al.*, 1993). In 2008 twelve TTM hybrids were divided by maturity into two groups, early and late, as determined by the difference in the days to anthesis . The five early hybrids (early TTM) were B73 x CML247, LH123 x CML333, LH123 x CML277, (LH132 x LH51) x CML242, and (LH132 x LH51) x CML427. The seven late hybrids (late TTM) were B73 x CML277, B73 x Mo18W, B73 x CML333, LH123 x Mo18W, LH123 x CML52, LH123 x Ki11, (LH132 x LH51) x Tzi3. Also included in the 2008 trial were two elite commercial grain hybrids classified as elevated fermentable carbon types, DKC61-69 and DKC64-79 (commercial grain*),* which also received an ear removal treatment (ear removed) on or near the brown silk stage (reproductive stage R3), and two commercial corn silage hybrids, W602S x LH244 and W605S x LH244, derived from the University of Wisconsin inbred lines produced using a tester line from Holden's Foundation Seeds (silage).

Plants were evaluated throughout the 2008 growing season for plant height, mean anthesis date, 50% silking date, anthesis-silk interval (ASI), and leaf area duration (LAD), based on 50% leaf senescence. Above-ground plant samples were collected in 2008 at two different sampling dates, September 24 (early TTM, commercial grain, ear

21

removed and silage, only), and October 26 (late TTM only). The selection of sampling

dates was predicated on the difference in maturity of the late TTM hybrids, and to allow

time for the late TTM to reach maximal biomass and sugar accumulation. Four whole

plant samples from each plot were pooled and then divided into leaf, stalk, reproductive

components (tassel, husk, cob) and grain. The fresh samples were weighed, shredded and

an approximately 300 g subsample was obtained. The subsamples were oven dried, at a

temperature of 158º F (70º C) to a constant weight to remove moisture, and reweighed to

calculate the biomass dry weight. Dried samples were ground using a Wiley Mill with a 2

mm screen, then a subsample of approximately 50 mg was analyzed for total non-

structural carbohydrates (sugar) using a modified Nelson Somogyi method (Heberer *et

al.*, 1985).

2.3.3 Germplasm Selection, Sampling and Measurements, 2009

The 2009 trial included seven TTM hybrids (2009 TTM), B73 x Mo18W, B73 x

Teosinte (*Zea* spp.), FR1064 x Mo18W, ILP x CML277, ILP x CML 52, ILP x Mo18W,

and TGI 02 (a proprietary stiff stalk modern inbred x NC358). Selection was based on

using a baseline hybrid from 2008 (B73 x Mo18W), and combining the common male to

females of differing maturity and heterotic groups, combining a common female (ILP)

with the male germplasm of the most promising hybrids from 2008 and a hybrid using a

modern elite grain inbred as the female. The inbred ILP (Illinois Low Protein) was

selected due to its high N utilization efficiency for dry matter production and starch

synthesis. It is one of the University of Illinois protein strains that are the result of long

term divergent selection for grain protein concentration and are considered unique within the maize germplasm (Uribelarrea *et al.*, 2007).

Ear shoots were covered with glassine bags before the silks emerged in 2009 to preclude pollination and grain formation allowing for better evaluation of the biological potential for sugar accumulation. Whole plant samples were obtained, on September 19, using the same collection process as 2008, with the following exceptions, five plants were harvested and plant components were not fractionated.

2.3.4 Ethanol Potential

Estimates for ethanol production potentials are based upon conversion factors, as set forth in the USDA report, The Economic Feasibility of Ethanol Production from Sugar in the United States (Shapouri 2006).

2.3.5 Statistical Analysis

Analysis of the data was conducted using JMP Version 8, (SAS Institute, Inc. Cary, NC, 2009) using the fit model function, full factorial. Significant effects were tested using Tukey's Honest Significant Difference (HSD) and the least significant difference (LSD) was calculated using the mean square error of the model. Year was not included in the model as treatments differed across years.

2.4 RESULTS

2.4.1 Different Hybrid Types, 2008 Results

Tropical maize hybrids, both early TTM and late TTM, produced taller, later maturing plants, and exhibited greater reproductive asynchrony through an extended anthesis – silking interval (ASI) than the commercial grain, ear removed or silage types (Table 2.1). Late TTM plants were the tallest and exceeded the height of the commercial grain hybrid by 60%. Average anthesis in the early TTM and late TTM occurred 16 days later than the temperate maize hybrids, while average silk emergence was 21 days later than the temperate hybrids. Supplemental N did not affect plant height, anthesis, silking or the ASI in any of the hybrid types, although commercial grain and silage hybrids both remained greener longer with the application of supplemental N (Table 2.1). Leaf area duration (LAD) varied for each of the hybrid types with the ear removed hybrid having the shortest LAD, followed by silage then commercial grain. The LAD of the early TTM hybrids exceeded commercial grain by more than 10 days, while late TTM had the longest LAD, remaining green until killed by frost on October 27. Nitrogen did not have an effect on the LAD of either the early or late TTM hybrids.

Although the TTM hybrids were taller than the other hybrid types (Table 2.1), the maximum biomass produced was similar to the commercial grain and silage hybrids and 50% greater than plants with their ears removed (Table 2.2). Supplemental nitrogen increased the total biomass of all hybrid types and altered the partitioning of this biomass among the plant components (Table 2.2). For all hybrid types, supplemental N was associated with a decrease in biomass partitioned to the stalk and leaf, and an increase in partitioning to grain. The greatest proportion of total biomass was partitioned to the stalk

24

in early TTM, late TTM, and hybrids with ears removed, while the grain was the main recipient of biomass in commercial grain and silage hybrids (Table 2.2). Without supplemental N, both early and late TTM hybrids partitioned twice the total biomass to the stalk compared to the commercial grain and silage hybrids, while ear removed plants partitioned three times as much. Although the commercial grain and silage types partitioned nearly twice the percentage of biomass to the grain component as early TTM and late TTM hybrids, nitrogen did not affect the percentage of biomass as grain. However, late TTM hybrids grown with supplemental N partitioned nearly twice the biomass to grain compared to those grown with no supplemental N (Table 2.2). The small percentage of biomass partitioned to grain in the ear removed plants occurred because some plants produced a second earshoot following removal of the apical earshoot.

It is evident that even with grain formation, TTM hybrids partition more biomass to the stalk and accumulate more sugar than commercial grain hybrids, and that supplemental nitrogen had a greater effect on the TTM hybrids in promoting grain formation and decreasing biomass partitioning to the stalk. Supplemental N also caused a 50% decrease in the concentration of stalk sugar in early TTM, late TTM, and ear removed hybrids, but did not affect commercial grain or silage hybrids (Table 2.3). Without supplemental N, early TTM and late TTM hybrids had over five times the concentration of stalk sugar than commercial grain hybrids, while those with ear removed had nearly ten times the concentration of stalk sugar. Despite the effect of N on sugar concentration, N had little impact on total sugar production of any hybrid type due to its additive effect on biomass production. However early TTM, late TTM and ear removed hybrids all accumulated between three and four times more sugar than the commercial

grain and silage hybrids (Table 2.3). Although the total biomass of the ear removed type was much less than the early TTM and late TTM plants, the total yield of sugar was similar due to nearly two times greater sugar concentration in the stalk of the ear removed plants. This finding suggests that the full potential for sugar accumulation can only be realized by preventing grain formation. However, early leaf senescence associated with ear removal constrains the potential for maximal stalk sugar accumulation by limiting the photosynthetic potential for assimilate production.

Commercial grain and silage both produced greater amounts of grain than the early TTM, late TTM, and ear removed hybrids, but also contained the least amounts of sugar. Both the early TTM and late TTM hybrids that produced grain also had decreased stalk sugar levels, particularly at the high level of N (data not shown). By comparing the grain yield across all hybrid types to their concentration of stalk sugar it is evident that grain yield negatively impacts sugar concentration at both levels of N (Figure 2.1 (a) and (b)).

2.4.2 Ear Shoot-bagged Temperate x Tropical Maize Hybrids, 2009 Results

Because 2008 data showed that grain production markedly decreased sugar levels, the plants in 2009 were ear shoot-bagged to prevent grain formation to more accurately determine the potential for sugar production. The seven genotypes exhibited similar growth and maturity patterns as the 2008 varieties, including late anthesis, delayed silk emergence and late maturity with all hybrids remaining green until frost on November 11, 2009 (data not shown).

The greater N supply increased biomass and total sugar production in all genotypes, while decreasing sugar concentration (Table 2.4). The elimination of grain formation by ear shoot-bagging resulted in twice the amount of stalk sugar when grown with low N, and an over threefold increase with supplemental N compared to the amount of sugar produced by the TTM hybrids in 2008 (Table 2.3). This increase in sugar content and concentration observed was despite the fact that the average amount of biomass produced by the ear shoot-bagged plants in 2009 was similar to the early TTM, late TTM, and commercial grain hybrids in 2008 (Table 2.2).

All genotypes responded to supplemental nitrogen by producing a greater amount of biomass, except FR1064 x Mo18W which produced nearly the same amount of biomass at both N rates (Table 2.4). The hybrid ILP x CML 52 produced the greatest amount of biomass and sugar with supplemental N, while at low N, ILP x CML 277 produced the most biomass and sugar. Total sugar produced by all genotypes was similar, with the exception of ILP X CML52 and TGI 02 which produced more sugar when grown with supplemental N than when grown with low N. The concentration of sugar was also similar across genotypes, with supplemental N decreasing sugar concentration in all genotypes, except ILP x CML 52 and B73 x Mo18W. Hybrid TGI 02, produced the least amount of biomass at low N, but exhibited the greatest response to N with a difference of 7.3 tons per acre between the two N rates with a similar difference in stalk sugar accumulation.

Variation between genotypes in response to nitrogen, biomass production, and stalk sugar content was evident. TGI 02 which has a modern temperate parent, exhibited the greatest response to supplemental N for sugar and biomass accumulation. The

27

variation in yields for sugar and biomass between genotypes, and the variable response to N suggests the potential to improve all plant components to meet the needs to produce a dedicated biofuel feedstock through modern plant breeding methods.

2.5 DISCUSSION

The findings of this study indicate TTM hybrids grown at midwestern latitudes of the U.S. are sensitive to photoperiod due to the genetics conferred by the tropical parent, resulting in delayed flowering and poor seed set (Table 2.1). This photoperiod sensitivity promoted an extended vegetative period, greater photosynthetic activity and enhanced assimilate accumulation in the TTM hybrids. Although the ear removed hybrids exhibited accelerated leaf senescence, TTM hybrids that were ear shoot-bagged remained green and retained stalk sugars which is contrary to previous findings that sink (i.e. ear) removal leads to earlier leaf senescence in commercial grain hybrids (Crafts-Brandner *et al.*, 1984). While supplemental N only extended the leaf area duration in the grain and silage hybrids, all hybrids produced a greater amount of biomass and grain, and partitioned a lower percentage of biomass to the stalks and leaves at the high N supply of 180 lbs/acre. This response to N fertilizer is consistent with reports that maize grown under N deficient conditions produces a reduced amount of grain and biomass due to a both less dry matter production and less partitioning to reproductive sinks (Uhart & Andrade, 1995).

Although TTM, silage and commercial grain hybrids differed in how they partitioned their biomass among plant components, they had similar total biomass yields within each N supply (Table 2.2). The TTM hybrids partitioned a greater portion of biomass to the stalk, whereas the commercial grain and silage hybrids partitioned the

28

majority of the plant biomass to grain. Other studies also indicate grain hybrids partition nearly 50% of the total biomass to the grain and 27% to the stalk (Pordesimo *et al.*, 2005). Related to the greater biomass partitioning to the stalk, TTM hybrids produced less grain and accumulated a greater concentration of stalk sugar than both the commercial grain and silage hybrids (Table 2.3), indicating an inverse relationship between grain production and stalk sugar concentration (Figure 2.1). This physiological response can be attributed to the translocation of assimilates from the stalk to the grain (Paponov & Engels, 2005). Conversely, the hybrids that had the ear shoot removed produced 50% less biomass than the other hybrid types, but had the same amount of sugar as the grain bearing TTM hybrids (Table 2.3). This finding suggests that the potential sugar production can only be achieved by preventing TTM hybrids from grain formation, which has been recognized in earlier studies (D'Ayala Valva & De Oliveira, 1980, Hume & Campbell, 1972, Van Reen & Singleton, 1952). As a result, the ear shoot-bagged TTM grown in 2009 accumulated significantly more stalk sugar (Table 2.4) than those grown in 2008 which produced some grain (Table 2.3). Although supplemental N decreased stalk sugar concentration, all but one of the ear shoot-bagged TTM hybrids yielded nearly the same total amount of total sugar per land area when grown at either high or low N supply (Table 2.4). A similar variable effect of N on growth and sugar levels has been reported for sweet sorghum also leading to comparable levels of total sugar (Smith & Buxton, 1993). Contrarily, the addition of N on sugarcane appears to markedly increase sugar yields. (Robertson *et al.*, 1996).

Although N did not affect the total amount of sugar produced by the ear shoot-bagged TTM hybrids, biomass yields increased with supplemental N (Table 2.4). The

biomass yields of the ear shoot bagged TTM, did not differ from that of the TTM, grain

and silage hybrids produced in 2008 (Table 2.2), which suggests a possible biological

biomass limit for maize. However, it also indicates that there is not a penalty to TTM that

would decrease biomass associated with enhanced stalk sugar accumulation when grain

formation is prevented. Biomass yield of the TTM hybrids when grown without

supplemental N was comparable to sweet sorghum yields produced with 125 lbs N /acre

in Iowa (Hallam *et al.*, 2001). Additionally, TTM yields when grown at high N were

comparable to commercial photoperiod sensitive sorghum hybrids produced under

irrigation and optimal nutrients in Texas (McCollum *et al*., 2005), as well as the

cumulative yields of two cuttings of sweet sorghum in Florida (Stanley & Dunavin,

1986).

Biomass, sugar content, sugar concentration and response to N varied between the

seven ear shoot-bagged TTM genotypes (Table 2.4), The genotype TGI02 that was

produced with a modern temperate parent exhibited the greatest response to N supply

with a marked difference in biomass and sugar yields depending upon the amount of N

supplied (Table 2.4). However, the overall moderate performance of TGI02 as a potential

biofuel hybrid indicates that crossing tropical with modern germplasm may not be an

effective means for TTM hybrid improvement. The hybrid ILP x CML 52 yielded the

greatest amount of biomass (nearly 13 tons/acre) and sugar (5,321 lbs/acre) when grown

with high N supply, but at deficient N produced the below average sugar yields and

unlike the other hybrids N did not decrease the concentration of sugar. In contrast, ILP x

CML 277 produced the greatest amount of biomass (10 tons/acre) and sugar (4,643

tons/acre) when grown with low N, and one of the highest yields of biomass when grown

at high N. However the reduced sugar concentration of ILP x CML 277 at high N

resulted in nearly equivalent total sugar yields regardless of the N supply rate. These

genotype differences indicate the potential for genetic improvement of TTM hybrids,

through tropical germplasm selection for nitrogen use efficiency, enhanced biomass and

elevated stalk sugar concentration (Tarter *et al.*, 2004). Other studies indicate substantial

variation among cultivars and populations for stalk sugar yields (Widstrom *et al.*, 1984)

and assert that the use of inbreds with abundant stalk sugar content will result in hybrids

with the same characteristic (Bertolini *et al.*, 1993). This initial evaluation of TTM

presented here tested a limited number of hybrid combinations with the majority

consisting of publicly available tropical and temperate germplasm, which have not been

selected for maximal total biomass or sugar accumulation. It is anticipated that both

biomass and sucrose yields can be rapidly be increased by applying the same strategies

used to select today's leading commercial grain hybrids, but by selecting for a different

set of traits, using biotechnology approaches, and identifying genes that can provide

benefits to the downstream saccharification and fermentation processes required for

ethanol production (Van Reen & Singleton, 1952; D'Ayala Valva *et al*., 1980; Widstrom

et al., 1984; Holland & Goodman, 1995; Tallury & Goodman, 1999).

Comparative ethanol estimates based on the grain, biomass and sugar data for the

different hybrid types (Tables 2.2, 2.3 and 2.4), indicate that TTM hybrids have the

potential to produce without supplemental N nearly the same theoretical yield of ethanol

per acre as commercial grain hybrids grown with a high rate of supplemental N (180

lbs/acre) when all components of both crops (sugar, grain and stover) are utilized in the

ethanol process (Table 2.5). These biofuel yields are also comparable to ethanol estimates

31

for sweet sorghum and photoperiod-sensitive sorghum indicating that TTM is a viable feedstock candidate for U.S. biofuel production (Propheter *et al.*, 2010, Smith & Buxton, 1993).

As an annual, widely adaptable crop requiring low fertilizer inputs, and possessing the unique attributes of both readily fermentable sugar and biomass, TTM can be a ready supplement to current ethanol processes and a feedstock for the long-range lignocellulosic biorefinery production model (BRDI 2009). Unlike ethanol produced from corn grain, which has an energy balance ratio of 1.4 to 1.5 (i.e. energy invested versus energy returned), the combined sugar and biomass of TTM would be analogous to the energy balance ratio of 8 to 9 assessed for sugarcane or sweet sorghum (Goldemberg, 2007). This greater energy efficiency of TTM counters the argument that maize as a species is a poor choice as a bioenergy feedstock. Because TTM requires less N inputs, it is on the periphery of the "food versus fuel" debate and the associated questions regarding environmental sustainability that have arisen around the use of corn grain for ethanol.

New feedstocks represent an opportunity to grow more biomass on any of the estimated annual 90 million-plus U.S. corn acres (USDA 2009) with projected decreased input requirements and costs than grain used for ethanol production. Near term implementation of TTM is possible as it is derived from corn, an annual crop that has a well established industry and infrastructure for support. Based on the genetic diversity and ample germplasm available, rapid advances can be made to genetically modify biomass, lignocellulosic and stalk sugar traits of TTM to meet current and future cellulosic ethanol processing and production requirements.

2.6 TABLES

Table 2.1 Influence of N supply and hybrid type on the growth, flowering and leaf area duration at Champaign, IL in 2008.

Hybrid type	Maximum Plant height		Anthesis		Silking		Anthesis-silking interval		Leaf area duration	
	Nitrogen supply (lbs N/acre)									
	0	180	0	180	0	180	0	180	0	180
	inches		DAP[b]		DAP		days		DAP	
TTM[c]-early	104	107	74	72	80	78	7	6	145	145
TTM-late	114	122	77	74	86	83	9	8	152	152
Commercial grain	72	76	59	57	62	59	3	2	135	140
Commercial grain ear-removed	74	78	59	57	62	59	3	2	122	125
Silage	73	69	60	59	62	62	2	3	130	135
LSD (*P*<0.05)	23		5		8		5		4	

ANOVA[a]

Source of variation										
Hybrid	***		***		***		***		***	
Nitrogen	NS		NS		NS		NS		***	
Hybrid x Nitrogen	NS		NS		NS		NS		***	

[a] *, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively; NS, not significant.
[b] DAP - Days after planting
[c] Temperate x tropical maize

Table 2.2 Influence of N supply and hybrid type on the production of total biomass, (aboveground portion) and its partitioning among plant parts at physiological maturity for plants grown at Champaign, IL in 2008. Sampling occurred at maximal biomass accumulation.

Hybrid type	Biomass		Stalk		Leaf		Grain		Repro[b]	
	Nitrogen supply (lbs N/acre)									
	0	180	0	180	0	180	0	180	0	180
	U.S. tons dry weight/acre		----------------------% of total biomass-------------------							
TTM[c] - early	5.6	7.7	46	37	23	20	20	31	12	13
TTM - late	6.4	10.4	50	40	21	16	16	30	14	14
Commercial grain	6.1	10.7	23	18	13	9	49	56	15	17
Commercial grain-ear removed	3.1	4.9	71	58	26	26	1	8	2	8
Silage	5.3	8.3	28	20	15	11	44	53	14	16
LSD (*P*<0.05)	2.7		15		7		18		4	

ANOVA[a]

Source of variation										
Hybrid	***		***		***		***		***	
Nitrogen	***		***		***		***		***	
Hybrid x Nitrogen	NS		NS		NS		NS		**	

[a] *, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively; NS, not significant.
[b] Repro – Reproductive component (cob, husk, tassel)
[c] Temperate x tropical maize

Table 2.3 Influence of N supply and hybrid type on the concentration and content of sugar in the stalk for plants grown at Champaign, IL in 2008. Sampling occurred at maximal biomass accumulation.

	Sugar concentration		Total sugar content	
	Nitrogen supply (lbs N/acre)			
Hybrid type	0	180	0	180
	% of dry weight		U.S. lbs dry weight/acre	
TTM[b] - early	11	6	1138	887
TTM - late	12	7	1403	1357
Commercial grain	2	2	280	440
Commercial grain-ear removed	19	13	1160	1165
Silage Hybrid	4	2	421	387
LSD (P<0.05)	6		599	

ANOVA[a]

Source of variation				
Hybrid	***		***	
Nitrogen	***		NS	
Hybrid x Nitrogen	NS		NS	

[a] *, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively; NS, not significant.
[b] Temperate x tropical maize

Table 2.4 Influence of N supply and genotype on total biomass (aboveground portion) and sugar levels in the stalk for seven ear shoot-bagged temperate x tropical maize hybrids grown at Champaign, IL in 2009. Sampling occurred at maximal biomass.

	Total biomass		Sugar concentration		Sugar Content	
	Nitrogen supply (lbs N/acre)					
Genotype	0	180	0	180	0	180
	U.S. tons dry weight/acre		% of dry weight		U.S. lbs dry weight/acre	
B73 x Mo18W	7.0	10.2	25	22	3522	4485
B73 x Teosinte	6.8	10.2	25	19	3351	3708
FR1064 x Mo18W	8.2	8.9	25	20	4156	3463
ILP x CML 277	9.9	12.1	24	18	4643	4403
ILP x CML 52	7.5	12.6	21	21	3089	5321
ILP x Mo18W	8.0	10.3	26	22	4058	4404
TGI 02	4.5	11.8	26	20	2335	4736
Mean	7.4	10.9	25	20	3593	4360
LSD (P<0.05)	2.2		4		950	

ANOVA[a]
Source of variation

Genotype	**		NS		**	
Nitrogen	***		***		***	
Genotype x Nitrogen	**		NS		***	

[a] *, **, *** Significant at the 0.1, 0.05 and 0.01 probability levels, respectively; NS, not significant.

Table 2.5 Influence of N supply and hybrid type on the potential ethanol yield for materials produced at Champaign, IL in 2008 and 2009.

	Plant Fraction							
	Sugar		Stover (bagasse)		Grain		Total	
	Nitrogen supply (lbs N/acre							
Hybrid type	0	180	0	180	0	180	0	180
	------------------Gallons per acre------------------							
TTM[a]–ear shoot bagged	258	295	678	983	0	0	936	1278
TTM-early	79	63	427	536	118	245	624	843
TTM-late	93	96	520	724	108	320	721	1139
Commercial grain	20	27	338	517	295	581	653	1125
Commercial grain-ear removed	75	75	281	414	5	63	362	551
Silage	27	20	310	431	231	430	568	881

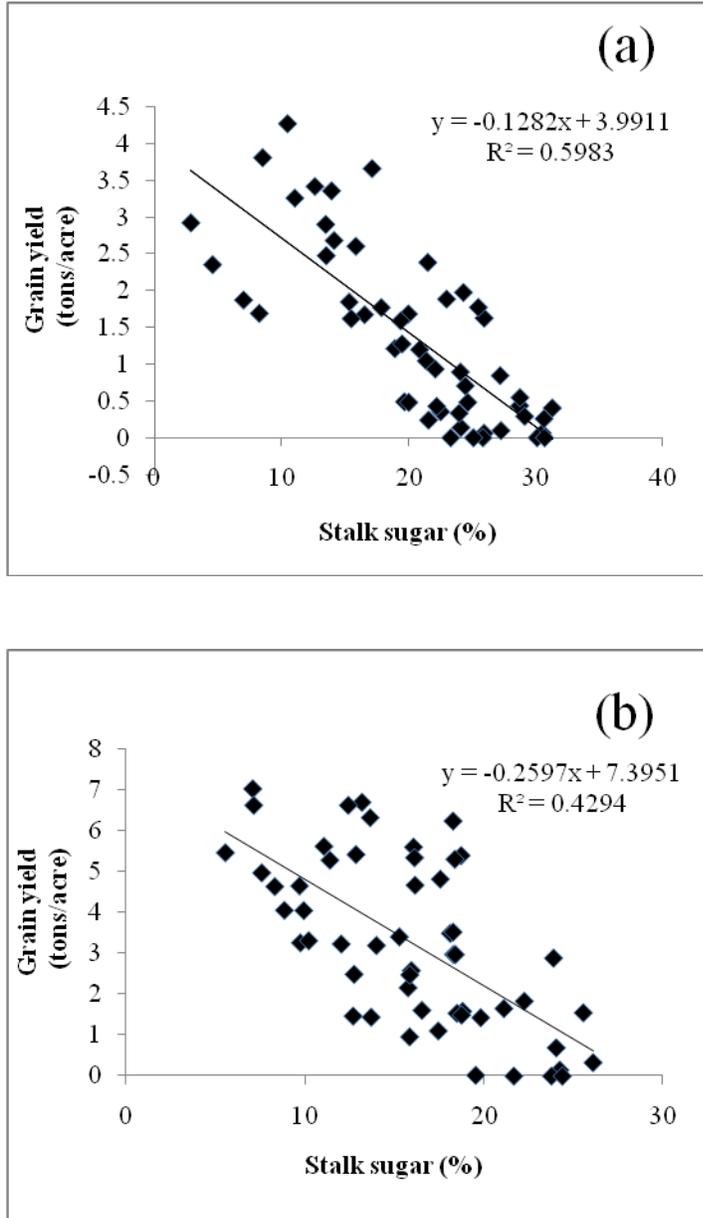[a] Temperate x tropical maize

2.7 FIGURES

Figure 2.1

The relationship between concentration of sugar in the stalk and grain yield at low N **(a)** and high N (180 lbs/acre) **(b)** for different hybrids grown at Champaign, IL in 2008 measured at physiological maturity. Hybrid types include tropical maize (early and late), commercial grain (with and without ear removal), and silage.

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