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Sugarcane tasseling under artificial photoperiod conditions as affected by nitrogen rate and temperature

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SUGARCANE TASSELING UNDER ARTIFICIAL PHOTOPERIOD
CONDITIONS AS AFFECTED BY NITROGEN RATE
AND TEMPERATURE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Plant, Environmental, and Soil Sciences

by
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ABSTRACT

Optimizing flowering in the LSU AgCenter's Sugarcane (*Saccharum* spp. hybrids) Breeding Program is an important step in the variety development program. The effect of nitrogen and ambient air temperature in pot cultured sugarcane were examined as a means of improving sugarcane flowering. The experiment was conducted on agronomic and reproductive traits of sugarcane at the Sugar Research Station, St. Gabriel, LA, on sugarcane genotypes subjected to artificial photoperiod regimes. The potting media consisted of equal parts of washed sand, Canadian peat moss, and a Commerce silt loam soil (fine-silty, mixed, nonacid, thermic aeric Fluvaquents). Early nitrogen (22.4-22.4-22.4 kg ha⁻¹) in addition to a high nitrogen potting media (>200 mg kg⁻¹) was necessary for adequate vegetative growth and stalk numbers for tasseling. Leaf macronutrient levels were examined at reproductive growth stages as affected by pre-photoperiod nitrogen fertilizers (22.4-22.4-22.4 kg ha⁻¹ and 0-22.4-22.4 kg ha⁻¹). Since tasseling in nitrogen and no-nitrogen treatments were 77% and 25%, respectively, the critical leaf nutrient level for nitrogen at the vegetative stage for sugarcane intended for tasseling should be 12.4 g kg⁻¹. A chlorophyll meter was used to collect chlorophyll readings from the same leaves that were sampled for plant analysis. The initiation stage was the only stage that both leaf nitrogen ($r = -0.34$) and chlorophyll meter readings ($r = 0.80$) showed significant associations. A chlorophyll index level (34.53) was developed as a maximum threshold level for sugarcane breeding genotypes at the initiation stage. Average daily maximum temperature for specific time intervals can affect sugarcane tasseling. A reduced regression model ($P=0.02$) for the overall tasseling regime indicated that the percent tasseling is expected to increase 4.19 percent when the May 30 – June 14 temperatures increase by one degree above 31.9 ° C, decrease by 4.36 percent

when the June 15- June 30 temperatures increase by one degree above 32.1° C, and decrease by 4.69 percent when the August 16 – September 10 temperatures increase by one degree above 33.1° C. These results help to explain the variation in tasseling percentages that have been encountered over the years when above average temperatures were experienced.

CHAPTER 1
LITERATURE REVIEW
HISTORICAL OVERVIEW

The oldest records of sugarcane cultivation date back to over a thousand years ago in India before the Christian era (Simon 1969). There is speculation that *Saccharum spontaneum*, the ancestral form of sugarcane, originated in India and may be the ancestor of *Saccharum barberi* and *Saccharum sinense*. What is now recognized as *Saccharum officinarum* probably originated in the gardens of New Guinea, probably as selections from *Saccharum robustum* and were spread through the South Pacific by prehistoric interisland travellers (Irvine 1983). Simon (1969) states that the introduction of sugarcane into Louisiana dates from 1751 when it was brought from Santo Domingo in the Dominican Republic and planted on the plantation of the Jesuit fathers, now a part of the city of New Orleans. In the late teens and early 20's, this "Noble" type of sugarcane, *Saccharum officinarum*, became infected with diseases and the yield of sugar decreased from an average of about 272,155 tonnes per year to a low yield of 42,638 tonnes in 1926. The failed resistance of noble sugarcane brought about the introduction of interspecific hybrid varieties from Java (POJ varieties) and India (Co. varieties).

Sugarcane as an industry in Louisiana rebounded with new varieties developed after the discovery that low temperatures were responsible for the low pollen fertility of most sugarcane varieties (Chilton and Paliatseas 1956). A sugarcane crossing station was established on Grand Isle, Louisiana, in 1948 by the Louisiana Agricultural Experiment Station. The site was later moved to the Baton Rouge campus of Louisiana State University and then relocated once again in 1982 to the LSU AgCenter's Sugar Research Station in St. Gabriel, Louisiana. In 1972, the United States Department of Agriculture expanded its sugarcane breeding program, and hybrid development was initiated from the basic breeding program at Houma, Louisiana (Dunckelman

and Legendre 1982). Sugarcane breeding programs at the LSU AgCenter and USDA, Houma, paved the way for the progress that the state has made throughout the 20th century and into the 21st century. Compared to a low sugar yield of 42,638 tonnes in 1926, Louisiana has the potential to produce approximately 1, 423, 990 tonnes of sugar (raw value) on a regular basis (Richard 2001).

Sugarcane Breeding

The Louisiana sugarcane industry is dependent upon a successful variety improvement program. The United States Department of Agriculture, the LSU AgCenter's Sugar Research Station, and the American Sugar Cane League all cooperate in the program (Simon 1969). The United States Department of Agriculture and the LSU AgCenter's Sugar Research Station each conduct commercial breeding programs that begin with the production of true seed at the sugarcane breeding facilities located in Canal Point, Florida and St. Gabriel, Louisiana respectively.

Advancement and selection within the breeding program requires a minimum of 12 years from the initial cross to the selection of hybrid plants suitable for cultivar release (Legendre and Burner 1997). The first stage or first year of the program is the "Crossing Stage". The personnel involved in this stage are responsible for the maintenance and care of the genotypes in can culture from the transplanting of eyepieces through the production of true seed. The multiple tasks of the crossing stage are performed in greenhouses and photoperiod houses. Much research in the past and present has focused on the control of tasseling for sugarcane genotypes. This is an essential part of the program for the breeding and selection of new varieties of sugarcane.

THE SUGARCANE TASSEL

A monocot, sugarcane belongs to the family Gramineae and the subfamily Panicoidea. Sugarcane is a perennial tropical grass that can be reproduced from true seed, from nodal buds, or, in certain species and a few interspecific hybrids, from rhizomes (Irvine 1983). All or many of the buds become reproductive, either in a flush of tasseling or over a more or less short period. Sugarcane is also a determinate plant. After seed setting, the axils that flowered can no longer continue growth. If all the axils become reproductive, then the plant dies, a process typical of obligate annuals. If only a proportion become reproductive, then the plant can regrow, although it may take some time to do so (Fisher 1999).

The grass flower is a reproductive organ, typically comprised of two tiny lodicules, three stamens and a unilocular ovary bearing two stigmas (Clayton 1990). The flower is enclosed by two bracts, a lemma on the outer side and a palea on the inner. By functional analogy with a perianth, the whole organ is commonly called a floret, even though the lemma is a modified leaf subtending the floral branchlet (Tran 1973), and the palea is a prophyll. Typically the florets are borne on opposite sides along a rachilla at the base of which are two empty scales called glumes. Glumes, rachilla and florets together comprise the spikelet. The spikelet is the basic unit of the inflorescence (Clayton 1990). In grasses, the total number of florets per inflorescence depends on the number of primary branches, and on the number of florets produced per primary branch. While there is only one spikelet per primary branch in ryegrass, basal branches of panicle grasses usually develop considerably more spikelets, and thus florets, than the terminal ones (Jeater 1956; Ryle 1966). Complete inflorescences of commercial hybrids in sugarcane have been estimated to contain 25,000 florets, but the number of fertile florets is always much lower (e.g. 3 % in NCO 310 and 33 % in B 7264) (Blackburn 1984). In sugarcane, the

inflorescence emerges above the mass of foliage. The main axis of the panicle arises almost imperceptibly from the terminal internode, and gradually narrows until it merges into the terminal rachis of spikelets. At the base of the panicle the primary branches are about 15 cm long, but shorter above. The secondary branches tend to arise in two rows, alternately along the primary branches and may carry tertiary branches. The ultimate branches bear the pairs of spikelets, one of which is sessile and the other on a stiff pedicel. Both spikelets have two florets, the lower one of which is sterile and represented by a delicate pointed lemma or third glume which is shorter than the glumes. The structure of both spikelets is similar, with a pair of hard boat-shaped glumes protecting the developing flowers. The upper floret of each spikelet has both male and female reproductive organs, with no lemma except in *S. spontaneum* and some of its hybrids. When present the lemma is a narrow scale with fine hairs at the top. At the base of the ovary opposite the palea are two short wedge-shaped lodicules. The three stamens with large bilobed anthers are in one whorl. The ovary is round, flattened on the ventral surface with a single anatropous ovule. The pistil has two long terminal styles each with a large brush-like feathery stigma, plum red in color.

The spikelets open during the night or early morning, beginning at the top of the panicle and progressing downwards and inwards over a period of one or two weeks. The lodicules swell and push the glumes apart and the stigmas are extruded (Blackburn 1984). Inflorescences are generally larger on early-formed tillers, and hence the inflorescence that appears first is generally the largest. Differences may be evident in inflorescence length or raceme length, depending on species characteristics (Hacker 1999).

After fertilization, there is a period of some three weeks or more during which seed maturation takes place. This is followed by the shedding of the ripe seed as the disintegration of

the panicle-branches takes place. On ripening, the lateral axes of the inflorescence disarticulate below the spikelets, breaking off at the nodes. The sustaining rachis segment, and also, curiously enough, the stalk of the pedicellate spikelet, remain attached to the sessile spikelet, while the other one (previously pedicellate) breaks free (Stevenson 1965). Collectively, the seed is commonly known as 'fuzz' (Blackburn 1984).

LSU AGCENTER SUGARCANE NUTRITIONAL PRACTICES FOR BREEDING GENOTYPES

Nutrition is important for floral induction in sugarcane breeding genotypes. After germination and some growth of the sugarcane parents, biweekly fertilization begins. Peters 2.4-22.4-22.4 kg ha⁻¹ water soluble general fertilizer is applied through the watering system with the aid of a Dosatron fertilizer injector at the rate that is equivalent to 400 mg kg⁻¹ each N-P₂O₅-K₂O. Plants with high C:N ratio tassel more easily than plants with a low C:N ratio. To raise the C:N ratio, nitrogen fertilization for all material is discontinued three weeks prior to the beginning of the artificial photoperiod regimes (Martin 1994). After six weeks of the initiation phase which starts with the beginning of the artificial photoperiod regimes, a reduced nitrogen fertilizer (8.9-26.9-26.9 kg ha⁻¹) is normally applied to the pot culture at a rate of 29.6 ml per pot. This fertilizer application occurs twice at one month intervals.

LSU AGCENTER ARTIFICIAL PHOTOPERIOD REGIMES

The artificial photoperiod regime chambers consist of six dark chambers that are used to manipulate day length to induce sugarcane genotypes to tassel. Three different artificial photoperiod regimes are used. All treatments differ with amount of darkness that the sugarcane breeding genotypes will receive. Difficult to initiate genotypes receive more treatment days than genotypes that are easier to initiate. The treatment differences offset the timing of tasseling for the different genotypes. This helps to alleviate congestion in the crossing house. The

photoperiod regimes begin on May 30 and end on September 10. All photoperiod regimes (time from artificial sunrise to natural sunset) are initiated with a minimum of 34 consecutive days of 12 ½ h of constant day length. After the initial constant 12 ½ h photoperiod regime, day length is shortened by one min per day. At the conclusion of the artificial photoperiod regimes, natural day length is 12 ½ h and decreasing (LaBorde et al. 2004). All genotypes are maintained in pot cultures. Pot culture (37.8 L) used for the LSU AgCenter's Sugarcane Breeding Program are transported from a greenhouse environment to an outdoor environment in early April of each year. The pot culture is placed on moveable rail carts in preparation of artificial photoperiod regimes.

FACTORS AFFECTING TASSELING IN SUGARCANE

Tasseling is of considerable practical importance in commercial sugarcane production since yields of sugar can be substantially reduced when plants tassel during commercial production. In breeding programs, control of tasseling is essential in the development of new sugarcane varieties. Therefore, a knowledge of the factors which regulate tasseling is valuable not only to the plant breeder, who must be able to control the timing of tasseling with precision, but also to the sugarcane grower, who may suffer financial loss as a result of heavy tasseling (Thompson 1984). Some of the factors that both inhibit and induce tasseling are photoperiodism, temperature, sugarcane age, moisture, and nutrition (Blackburn 1984).

Photoperiodism

Photoperiodism can be defined as a response in plants to the seasonal variation of daylength (Ting 1982). In grasses, research has shown that photoperiodism is often related to the timing of other climatic variables such as daylength, temperature, rainfall, and humidity that can inhibit seed production (Loch et al.1999). The full range of tropical and subtropical forage

grasses is adapted across a wide diversity of environmental conditions so that no single area is ideal for producing seed of all species or cultivars. The principal factors known to control the transition from vegetative to reproductive growth in grasses and legumes are photoperiod and temperature (Aamlid et al. 1999). To control daylength and temperature in the temperate sugarcane growing regions throughout the world, artificial photoperiod regimes have been developed for specific locations. An improved knowledge of sugarcane breeding and selection highlights the need for making planned crosses for special traits such as resistance to diseases and insect pests, or high early sucrose for the commercial sector. Parental genotypes tassel during specific periods, and because genotypes may tassel at different times, they cannot be crossed easily. With artificial photoperiod regimes, the control of tasseling has made possible planned rather than opportunistic sugarcane crosses (Nuss and Berding 1999). Sugarcane is generally regarded as a short-day plant, although certain varieties will only tassel when the photoperiod occurs within a very narrow range; characteristic of intermediate or middle-day plants (Thompson 1984). Plants that will only initiate if exposed to days shorter than a specified length are called short-day plants (Fisher 1999). Tasseling in sugarcane is initiated by a small decrease (30 to 60 sec per day) in daylength from about 12 h and 30 min (Berding 1995; Moore and Nuss 1987). Artificial photoperiod regimes are often achieved by the construction of dark chambers in which the sugarcane breeding genotypes can be rolled in and out at certain times to achieve the desired amount of daylength. After subjecting the sugarcane breeding genotypes to an allotted number of inductive cycles of the artificial photoperiod regimes, initiation of the tassels will occur. In the early days of research on photoperiod, not much was known about the factors that control tasseling. Most of the work concentrated on finding the proper daylength for tassel induction (Abou-Salama 1990). While researchers agree that photoperiod is the key factor

influencing the behavior of sugarcane with regard to tasseling, they also agree that temperature, age of cane, soil moisture, and soil fertility at the time of inductive daylengths interact with the photoperiod to enhance, retard, or prevent transformation of the sugarcane apices from vegetative to reproductive growth (Dunckelman and Blanchard 1974).

Temperature

Temperature is another important factor that can influence tasseling in plants. Tasseling may be adversely affected in subtropical and temperate areas where the daily nighttime lows are below a certain critical temperature. Temperatures below 18.3° C during the initiation phase of sugarcane can prevent tasseling if these temperatures continue for six nights or more (Thompson 1984). In Louisiana, low temperatures are very common during the months when sugarcane tassel initiation would occur naturally and this has constrained the LSU AgCenter's Sugarcane Breeding Program to the use of artificial photoperiod regimes to initiate and synchronize tasseling. The artificial photoperiod regimes take place during a more conducive climate for sugarcane tasseling which coincides with Louisiana's summer months. The duration of the summer months are necessary to develop a phase change, from the vegetative to the reproductive, in sugarcane. Although the time frame appears optimum for Louisiana, little is known about the effect of temperatures in June, July, August, and September on tassel initiation and seed set.

The artificial photoperiod regimes used by the LSU AgCenter were developed in the 1950's (Gravois and Bischoff 2001). The earliest evidence of high temperature suppression on the initiation of sugarcane occurred in 1967 (Clements and Awada 1967). Night temperatures above 23.8° C and below 21.1° C reduce the number of initials (Brett 1946). A maximum seed set per tassel or per g of fuzz is achieved under temperatures between 26.6-29.4° C (day) and

21.1-23.8° C (night) (Paliatseas 1976). High night temperatures can be mitigated by the use of exhaust fans in photoperiod dark chambers. In facilities lacking appropriate lighting where sugarcane is rolled outside during the day, daytime temperatures are not controlled and left to fluctuate with environmental conditions. Daytime temperature above 32.2° C during the start of initiation inhibited tasseling in sugarcane (Moore and Nuss 1987).

The adaptive function of the specialized C₄ pathway appears to be the ability to retain carbon dioxide entering the plant in daylight under virtually any conditions (Laetsch and Kortschak 1971), an adaptation which could have advantages under many stress situations. Sugarcane uses a C₄ mechanism of photosynthesis similar to other tropical grasses (Anonymous 2004). Because the optimum temperature for C₄ plant growth and development is 35° C, the LSU AgCenter's Sugarcane Breeding Program strives to maintain this temperature throughout its vegetative life cycle. From germinating in the greenhouse to transplanting into pot culture, greenhouse heaters and normal daytime temperatures in the months of October through April help to maintain an optimum temperature for the vegetative growth of sugarcane breeding genotypes. Sugarcane breeding genotypes are moved from greenhouse conditions to ambient conditions in April of each year. At this stage of growth (artificial photoperiod regimes), certain studies have shown an inverse relationship of sugarcane initiation and high temperatures. Other factors which enhance fertile tiller survival and inflorescence size in grasses include moderate temperatures (Ryle and Langer 1963a,b; Ryle 1965; Heide 1982) and photoperiods slightly longer than the critical ones (Heide 1987, 1988; Ryle and Langer 1963b; Ryle 1965).

Sugarcane Age

The age of sugarcane may promote or inhibit floral initiation. Sugarcane displays a 'ripeness-to-flower', in common with other plants, in that stalks which are too young cannot be

induced to tassel. There are genotype differences in the length of the juvenile phase (Mangelsdorf 1946, 1953). In a young stool with stalks of many sizes only one or two of the largest shoots may tassel, so the term ‘percentage of tasseling stalks’ is an expression of the number of stalks which reach the ‘ripeness-to-flower’ stage by the critical date for tassel initiation (Thompson 1984). Calder (1966) defined juvenility as a phase where plants are insensitive to environmental conditions which later, in the mature or adult phase, promote tasseling. Applied to grasses, this definition only has relevance for seedlings yet, Calder (1963, 1964, 1966) also stated that it is uncertain whether juvenility in grasses is a property of every individual tiller, only of main shoots, or of the plant as a whole. It is generally assumed that the end of the juvenile phase and the beginning of the photoinductive stage coincides with the development of two to four mature internodes at the base of the stalk (Burr et al. 1957; Clements and Awada 1967; Coleman 1969; Julien 1973). Older tillers also develop more florets per primary branch than younger ones (Ryle 1966; Hill and Watkin 1975; Colvill and Marshall 1984).

Moisture

In general, soils for seed production should be well drained and should have a reasonable moisture-holding capacity as a buffer against fluctuations in rainfall. Native soil fertility is usually less important, as nutrient deficiencies can be corrected by addition of fertilizer (Loch et al. 1999), although with adequate moisture, grass seed crops can make greater use of available nitrogen than under dry conditions. Actively growing crops make greater demands on water and other nutrients. Variable soil moisture conditions are therefore likely to cause greater fluctuations in seed yield at higher nitrogen rates (Chadhokar and Humphreys 1973), particularly in moisture-sensitive species. Low rainfall has also been shown to reduce the intensity of

tasseling in native soils (Alexander 1924; Pereira et al. 1983; Yeu 1980). Researchers also showed that in areas where photoperiod and temperature seldom inhibit tasseling, the variation in intensity of tasseling between years was primarily the result of differences in annual rainfall (Moore 1987; Yeu 1980). Low moisture during the initiation period reduces tasseling and a quantitative relationship has been found between amount of irrigation applied and the extent of tasseling (Berding 1995; Gosnell 1973). Adequate moisture is critical for induction, tassel development, time of tassel emergence and seed set (Moore and Nuss 1987).

Nutrition

In crop species where fruits, seeds, and tubers represent yield, the effects of mineral nutrient supply on yield response curves are often a reflection of sink limitations, imposed by either a deficiency or an excessive supply of mineral nutrients during certain critical periods of plant development, including tassel induction, pollination, and tuber initiation. These effects can be either direct (as in the case of nutrient deficiency) or indirect (e.g., effects on the levels of photosynthates or phytohormones) (Marschner 1997). Normally at the time of tassel initiation, sugarcane is growing rapidly, all excess nitrogen has presumably been leached from the sand in the potting soil and the stalks have a minimum of 6 mature internodes (Nuss 1980). For maximum tasseling, sugarcane must be growing vigorously before induction. However, high levels of nitrogen, especially at the time of induction, consistently inhibit tasseling (Burr 1950; Clements and Awada 1967; Stevenson 1965; Gosnell 1973; Allam et al. 1978; Nuss 1977). Because nitrogen plays such a central role in determining grass seed yield, a key decision is how much nitrogen fertilizer to apply and when this should be done.

There have been a number of experiments to determine optimum rates of nitrogen for various species and cultivars. As a general guide, rates near the optimum lead to minimal

lodging in most grasses; and the growing crop should retain darker green leaves until about inflorescence emergence, after which it lightens in color as it matures (Loch et al. 1999). Light intensity and nitrogen also affect inflorescence size in many species (Ryle 1964, 1966). During the early stages of clonal development, nitrogen is important because nitrogen promotes vegetative growth and tillering important for the growth of the breeding genotypes. The younger the crop, the lower the quantity of nitrogen required to inhibit tasseling. There are large differences among genotypes; a few are so sensitive that tasseling never occurs under normal levels of fertilization (Gosnell 1973). The ability of older plants and certain genotypes to use nitrogen without inhibiting tasseling may be related to the carbon/nitrogen ratio (Chang and Huang 1980). Tassel development and emergence are reported to be inhibited by nitrogen (Allam et al. 1978). High levels of nitrogen, particularly at the time of initiation, may inhibit or delay tasseling. The extent of the inhibition is affected by the age of the sugarcane, the genotype and the availability of water. Tasseling at Mount Edgecombe, South Africa was delayed by 25 days because of excessive amounts of nitrogen in the soil (Nuss and Berding 1999). In a fertilizer experiment in Queensland, it was found that the percentages of tasseling stalks in plots supplied with 0, 224, and 448 kg of ammonia sulphate per hectare were 71, 54 and 11, respectively (Anonymous 1940).

Studies of the effects of nitrogen on sugarcane tasseling have shown that nitrogen influences both the initiation and the induction phases of tasseling (Nuss and Berding 1999; Nuss 1977). In addition, the effect of nitrogen on seed production has also been researched with limited findings. Much of the research on nitrogen has been done only on certain species of grasses with general findings. Fertilizer nitrogen increases seed yield mainly through an increase in the number of inflorescences, although other yield components (e.g. inflorescence size) also

respond to a lesser extent in some grasses (Loch et al. 1999). In most species where seed yield increased with increasing levels of applied nitrogen, this was mainly attributable to slight but often non-significant increases in number of fertile tillers per unit area and seeds per fertile tiller (Hebblethwaite et al. 1980). Similar reasons for an increase in seed yield to increasing levels of nitrogen were shown by Hebblethwaite and Ivins (1977). In all species, level of nitrogen application had no significant effect on individual seed weight possibly because seed is buffered against adverse conditions by the considerable potential of the crop to translocate assimilates from stem and leaf reserves when conditions for current photosynthesis are poor (Hebblethwaite et al. 1980). The main effect of fertilizer nitrogen on tropical/subtropical grass seed crops is to increase seed yield via increased inflorescence density (Humphreys and Riveros 1986; Hill and Loch 1993). In perennial ryegrass, the number of florets per spikelet and hence per unit area was decreased. The number of seeds per unit area was also decreased, but to a lesser extent, so that the percentage seed set was increased (Hebblethwaite and Ivins 1977).

The phosphorus requirement for optimal growth is in the range of 3-5 g kg⁻¹ of the plant dry matter during the vegetative stage of growth. The probability of phosphorus toxicity increases at contents higher than 10 g kg⁻¹ in the dry matter (Bell et al. 1990). Photosynthesis is inhibited by phosphorus deficiency, and the photosynthetic efficiency per unit of chlorophyll is much lower in phosphorus deficient leaves (Lauer et al. 1989). Although total respiration is not altered in phosphorus deficient roots, the proportion of alternative respiration increases from about 40-50% in phosphorus-sufficient to 80-90% in phosphorus-deficient roots, a shift which can be reversed within a few hours after resupply of phosphorus (Rychter and Mikulska 1990).

Despite these adaptive responses in increasing phosphorus acquisition by roots, not only is shoot growth rate retarded by phosphorus limitation but also the formation of reproductive

organs. Tassel initiation is delayed (Rossiter 1978), the number of flowers decreased (Bould and Parfitt 1973), and seed formation restricted in particular (Barry and Miller 1989). Premature senescence of leaves is another factor limiting seed yield in phosphorus-deficient plants.

Equilibrium exists between the soluble, exchangeable and fixed forms of potassium. Like all equilibriums, a change in the concentration of any one of the constituents will cause a shift towards stabilization. For example, depletion of the soluble potassium in the soil by the plant and soil microorganisms will cause a release of exchangeable potassium, which in turn, will cause the slow release of fixed potassium. This equilibrium is desirable because adsorbed and fixed potassium, which are not readily leached from the soil, can be made available to plants (Yadava 1993). Low potassium levels in the leaves have been correlated with a high proportion of sterile female flowers in *Solanum sisymbriifolium* (Wakhloo 1975a,b).

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CHAPTER 2
EFFECT OF NITROGEN ON SUGARCANE TASSELING
UNDER AN ARTIFICIAL PHOTOPERIOD REGIME

INTRODUCTION

At the LSU AgCenter's Sugar Research Station in St. Gabriel, Louisiana, artificial photoperiod is an integral tool in the sugarcane variety development program to induce the tasseling of sugarcane (*Saccharum spp.*). The necessity of an artificial photoperiod regime in Louisiana is due to the natural low temperatures (< 15.6° C) which coincide with the normal sugarcane tassel initiation phase in St. Gabriel, Louisiana (latitude 30° 15' N, longitude 91° 05' W). Tassel initiation is inhibited by cold temperatures. Artificial photoperiod aids in the initiation of sugarcane breeding genotypes by advancing the initiation phase to an earlier, more temperature conducive environment (> 15.6° C and < 32.2° C). With artificial photoperiod regimes, the control of tasseling has made possible planned rather than opportunistic sugarcane crosses that are required for a successful sugarcane breeding program (Nuss and Berding 1999). Artificial photoperiod has gained recognition not only in temperate climates where it is necessary, but also in tropical/subtropical climates where sugarcane tasseling occurs naturally. Therefore, knowledge of the factors that regulate tasseling is valuable to the plant breeder who must be able to control the timing of tasseling with precision (Thompson 1984). Over the years, artificial photoperiod regimes have enabled the LSU AgCenter sugarcane breeders to make sugarcane crosses to provide improved germplasm through selection.

In Louisiana, sugarcane breeding genotypes that undergo any type of artificial photoperiod regimes must be planted in pot culture. Pot culture is essential to move the sugarcane breeding genotypes in and out of the artificial photoperiod house. Unlike most tropical breeding programs such as in Australia, Columbia, Cuba, and Taiwan that can grow

sugarcane breeding genotypes both naturally and under artificial photoperiod regimes for hybridization purposes (Nuss and Berding 1999), the LSU AgCenter's Sugarcane Breeding Program relies entirely on pot culture. It is in this pot culture that nutrition plays an important role in the growth of sugarcane. Inadequate nutrition may adversely affect the successful growth of sugarcane breeding genotypes in preparation for and during artificial photoperiod induction.

Early research concentrated on estimating the proper daylength for tassel induction (Abou-Salama 1990). While researchers agree that photoperiod is the key factor influencing the behavior of sugarcane with regard to tasseling, they also agree that temperature, age of sugarcane, soil moisture, and soil fertility at the time of induction interact with photoperiod to enhance, retard, or prevent sugarcane tasseling (Dunckelman and Blanchard 1974). Because sugarcane must be grown vigorously for maximum tasseling (Moore and Nuss 1987), high levels of nitrogen during the initiation phase may reduce or delay tasseling (Van Dillewijn 1952; Clements and Awada 1967; Nuss and Berding 1999), whereas too little nitrogen may affect tasseling intensity, flower size, and seed set (Brunkhorst 2001).

Although nitrogen is required in greatest amounts, the optimum fertilizer rate for pot culture of sugarcane breeding genotypes prior to and during the time of induction is largely unknown. Nitrogen serves as a constituent of many plant cell components, including amino acids and nucleic acids (Taiz and Zeiger 2002) that are needed by the plants. The effect of nutritional status on tasseling of sugarcane at the LSU AgCenter's Sugar Research Station is based on generalizations that the ability of older plants and certain breeding genotypes to use nitrogen without inhibiting tasseling may be related to the carbon/nitrogen ratio (Chang and Huang 1980). Repeated fertilizations are required because of the leaching effect of constant watering. Constant watering is needed because potted soil media tends to dry out quickly. When

more rapid growth of the plants is desired, the rate of fertilizer is doubled (Dunckelman and Legendre 1982).

This research was performed to determine the impact of nitrogen fertilization prior to and during the time of induction of sugarcane breeding genotypes grown in pots. The objectives of this study were: (i) to investigate the effect of nitrogen on tasseling and on agronomic and reproductive traits, and (ii) to develop an optimum nitrogen fertilizer treatment regime for the LSU AgCenter's Sugarcane Breeding Program.

MATERIALS AND METHODS

Design of Experiment

A two-year (2003-2004) fertilizer experiment was conducted on three sugarcane genotypes used for hybridization at St. Gabriel, Louisiana (latitude 30° 15' N, longitude 91° 05' W). The three sugarcane genotypes were LCP85-384 (Milligan et al. 1994), LCP86-454 (Martin et al. 1996), and HoCP85-845 (Legendre et al. 1994). All three of these genotypes were formerly released as commercial sugarcane varieties. All three sugarcane genotypes contain qualities of high genetic value that make them important for breeding and are classified as “easy-to-induce” genotypes (LaBorde et al. 2004).

Clonal propagation began in October each year for the subsequent breeding seasons. The sugarcane genotypes were propagated into styrofoam trays that contained Metro Mix 350 horticultural mix (Sun Gro, Canada). In addition to the horticultural mix, a supplement consisting of 3 g Peter's fritted trace elements in 453.60 g dolomitic limestone and 907.2 g of superphosphate was mixed with each 0.08 m³ bag of Metro Mix 350 (Martin 1994). Liquid fertilizer treatments (22.4-22.4-22.4 kg ha⁻¹) were applied on a biweekly basis until the sugarcane genotypes were transplanted in January 2003 and January 2004 into large pot culture (37.8 L).

The soil mixture consists of equal parts of washed sand, Canadian peat moss (Sun Gro, Canada), and a Commerce silt loam (fine-silty, mixed, nonacid, thermic aeris Fluvaquents) soil. The Canadian peat moss in the media improves moisture-holding capacity, the relationship of air to water, and the ability to physically manage the mixture. Both sand and Canadian peat moss create and enhance a very desirable plant growing media (Donahue et al. 1983) with the addition of commerce silt loam. The major chemical effect of soil organic matter (SOM) in most soils is that it contributes 20 to 80% of the cation exchange capacity (CEC) that is proportionate to the soil pH (Sylvia et al. 1998). Genotypes were maintained in pot culture for the remainder of the experiment. It is in this 37.8 L pot that the sugarcane breeding genotypes complete their life cycle throughout artificial photoperiod regimes that LSU uses for tassel initiation (LaBorde et al. 2004).

The experimental design for each year was a completely randomized design that included 12 fertilizer treatments (Table 1). The fertilizer treatments were divided into two different timeframes, pre-photoperiod and post-photoperiod treatments. The pre-photoperiod and post-photoperiod treatments coincide with the vegetative and reproductive phases, respectively. Each experimental unit consisted of one 37.8 L pot with a surface diameter of 40.6 cm and a height of 36.8 cm. Each pot contained two sugarcane setts that were allowed to tiller. Once two tillers were established for each primary stalk, all other tillers were removed to provide six potential sugarcane stalks per pot. Although tillering is desirable to produce an equal number of stalks in each pot, many pots with certain treatments failed to produce adequate tillers. The sampling unit for each experimental unit consisted of sugarcane stalks.

Fertilizer Treatments

The fertilizer treatments for the study consisted of two different formulations needed to evaluate the effect of nitrogen on sugarcane breeding genotypes. For both formulations, phosphorus and potassium are calculated for the oxide basis (N-P₂O₅-K₂O). The liquid formulation (22.4-22.4-22.4 kg ha⁻¹) is the standard formulation that has been used for many

Table 1. List of fertilizer treatments. Phosphorus and potassium formulations are calculated from oxide basis (N-P₂O₅-K₂O) not elemental basis.

Treatment	Pre-photoperiod Treatments †	Post-photoperiod Treatments‡
1	22.4-22.4-22.4	0-22.4-22.4
2	22.4-22.4-22.4	2.8-22.4-22.4
3§	22.4-22.4-22.4	5.6-22.4-22.4
4	22.4-22.4-22.4	11.2-22.4-22.4
5	22.4-22.4-22.4	22.4-22.4-22.4
6	22.4-22.4-22.4	44.8-22.4-22.4
7	0-22.4-22.4	0-22.4-22.4
8	0-22.4-22.4	2.8-22.4-22.4
9	0-22.4-22.4	5.6-22.4-22.4
10	0-22.4-22.4	11.2-22.4-22.4
11	0-22.4-22.4	22.4-22.4-22.4
12	0-22.4-22.4	44.8-22.4-22.4

† For 37.8 L container, the pre-photoperiod treatments (1-6) was applied as liquid at a rate of 3.5 L per 37.8 L container; the pre-photoperiod treatments (7-12) was applied as granular formulation at a rate of 29.6 ml per 37.8 L container.

‡ For 37.8 L containers, the post-photoperiod treatments were applied as granular at a rate of 29.6 ml per 37.8 L container.

§ Treatment 3 represents the standard fertilizer formulations and rates that the LSU AgCenter uses in the photoperiod stage of its sugarcane breeding program.

years. The source for the liquid formulation (Scotts Professional Solutions, Ohio) lacked a comparable no-nitrogen liquid formulation. As a substitute for the no-nitrogen liquid formulation, a granular fertilizer was used to compare the effects of nitrogen on sugarcane breeding genotypes.

Pre-photoperiod treatments consisted of a liquid formulation of Peters 22.4-22.4-22.4 kg ha⁻¹ for the nitrogen applied treatments and a granular fertilizer formulation consisting of 0-22.4-22.4 kg ha⁻¹ for the no-nitrogen treatments. The liquid formulation was applied at a rate of 3.5 L per 37.8 L container while the granular formulation was applied at a rate of 29.6 ml per 37.8 L container. These fertilizer treatments were applied on a biweekly basis beginning on the first of February for each year and ending three weeks prior to the beginning of the artificial photoperiod regime on May 30. There were approximately eight fertilizer applications consisting of both formulations for the pre-photoperiod treatments.

All post-photoperiod treatments consisted of granular formulations ranging from 0 kg ha⁻¹ to 44.8 kg ha⁻¹ nitrogen rates with a constant phosphorus and potassium rate of 22.4 kg ha⁻¹. The post-photoperiod treatments were applied six and ten weeks after the artificial photoperiod regime began for a total of two applications during 2003 and 2004. Each post-photoperiod treatment was applied as a granular rate of 29.6 ml per 37.8 L container. The granular fertilizer formulations (pre-photoperiod and post-photoperiod treatments) were thoroughly mixed by hand in a tub. The sources of nitrogen, phosphorus, and potassium are ammonium nitrate (34%) N, triple superphosphate (46%) P₂O₅, and potash (60%) K₂O. Vermiculite was used as filler to calibrate correct formulation for some of the treatments.

Data Collection

Soil samples were taken prior to fertilization of the experiment to identify soil-nutrient levels. The soil samples were taken from several experimental units and mixed for a representative sample in each year. The soil test was done at the LSU AgCenter's Soil Testing and Plant Analysis Laboratory (STPAL) in Sturgis Hall. A chemical analysis of the soils is listed in Table 2 along with LSU AgCenter's fertilizer recommendations for a field grown plant cane crop. LSU fertilizer recommendations are based upon vegetatively growing sugarcane and should not be confused with sugarcane while in a reproductive phase.

Data collected from this study consisted of traits measured at four different stages of the sugarcane life cycle (Figure 1). The first stage was prior to the artificial photoperiod regime which was referred to as the vegetative stage; the second stage or initiation stage was equivalent to the first post-photoperiod treatment application which began six weeks subsequent to the beginning of the artificial photoperiod regime; the third stage or the elongation stage was at the first visible sign that initiation had occurred; and the final stage was anthesis. The vegetative and initiation stages occurred at the same julien date for each genotype (Figure 1; Stage 2); the elongation stage represents the first definite sign of initiation; and the anthesis stage represents the date of first tassel for each genotype. The initiation stage represents the transformation from the vegetative stage of growth to the reproductive stage of growth. Upon initiation, stalks continue to the elongation stage, followed by the anthesis stage. If initiation does not occur, then the sugarcane plant remains in the vegetative stage (nontasseling developmental pathway). At each stage prior to anthesis, traits measured included stalk number, stalk height, and stalk diameter. Between the elongation stage and the anthesis stage, the date of early flag leaf stage was recorded to determine the fertilizer treatment effects on the duration of this intermittent

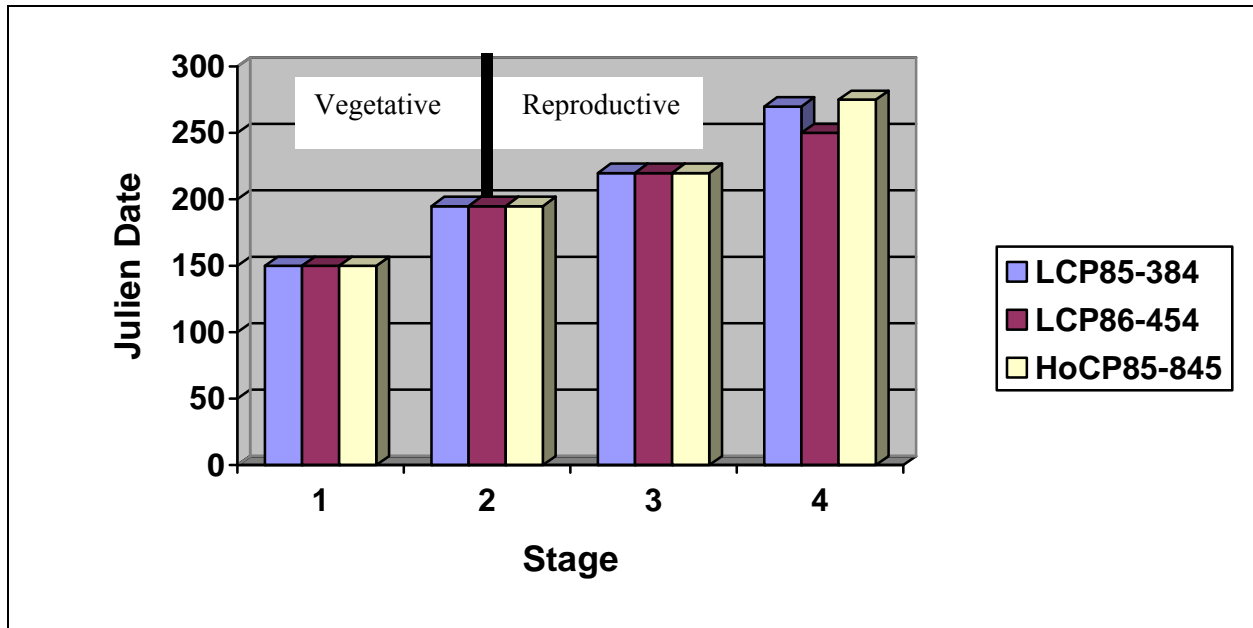


Fig. 1. Visual of different stages of tasseling developmental pathway and three genotypes. Stage 1 is the vegetative stage, stage 2 is the initiation stage, stage 3 is the elongation stage, and stage 4 (†) is the anthesis stage. The line on stage 2 signifies that it is a transition stage between vegetative growth and reproductive growth.

† Stage 4 consists of differences in Julian date because each genotype tasseled at a different average date.

reproductive stage. At anthesis, tassel number, inflorescence length, and peduncle diameter were measured.

Statistical Analysis

The data were analyzed at two different time frames to determine the relationship between nitrogen and sugarcane breeding genotypes in a vegetative stage and a subsequent reproductive stage. The experimental design was a completely randomized design. The dependent variables were stalk number, stalk height, and stalk diameter for the vegetative stage. For the reproductive stage, the dependent variables were tassel number, inflorescence length, flag leaf date (date that stalk entered flag leaf stage), flag leaf duration (number of days that stalk is in flag leaf stage), anthesis date, and peduncle diameter.

For this study, the data were analyzed with the following model:

$$Y_{ijkl} = \mu + \alpha_i + \pi_j + \alpha\pi_{ij} + Y_k + e_{ijkl}$$

Where Y_{ijkl} was the observed response of the i fertilizer treatment (α) for the j genotype (π) in k year (Y); μ is the general mean, $(\alpha\pi)_{ij}$ is the interaction of treatment with genotype, and e_{ijkl} is the normally distributed random experimental error.

The data were analyzed using the Proc Mixed procedure of SAS (Freund and Wilson 1993). Treatment was tested for significance by using the (genotype x treatment) interaction since year was considered a random variable. Because year was considered a random variable, the treatments were averaged across the years. Least square means were calculated, and mean separation was performed with a significance level of $P \leq 0.05$. Letter groupings were converted using the PDMIX800 macro in SAS (Saxton 1998).

RESULTS AND DISCUSSION

Soil Test

For 2003 and 2004, the pH level for each potting mix was low (Table 2). Availability of most plant nutrients is usually best in a soil with a pH of 5.8-7.0. Commercial production yield decreases can occur when pH falls below 5.5 on silt loam and sandy loam soils and below 5.2 on clay loams and clays (Faw and Funderburg 1995). When the pH falls below 5.8 on sandy loam or silt loam soils or below 5.2 on clay loam or clay soils, lime is recommended to reduce soil acidity. Yield decreases regarding pH for reproductive sugarcane in pot culture have yet to be studied. Plants in pot culture can experience excessive leaching. Nutrient availability in pot culture is derived from the potting soil mixture (1/3 field soil, 1/3 sand, 1/3 organic matter) in the form of nutrient residual and the addition of required nutrients. Nutrients for pot culture are limited and may be affected by the pH of the soil but little attention has been given to this

Table 2. Results from chemical analysis of the potting media for sugarcane breeding genotypes. The interpretations for 2003 and 2004 are based on sugarcane field production requirements.

	2003	2003	2004	2004
	St. Gabriel	Interpretation	St. Gabriel	Interpretation
Soil Texture†	Loamy sand		Loamy sand	
pH	4.77		4.63	
Nitrogen (g kg ⁻¹)	.046 (460 mg kg ⁻¹)		.046 (460 mg kg ⁻¹)	
Phosphorus, mg kg ⁻¹	11.40	Very low	20.82	Very low
Potassium, mg kg ⁻¹	29.72	Very low	61.26	Very low
Sodium, mg kg ⁻¹	30.10	Optimum	28.56	Optimum
Magnesium, mg kg ⁻¹	137.08	High	198.74	Medium
Calcium, mg kg ⁻¹	408.85	Very low	869.96	Low
Carbon	1.16		0.07	
N Recommendation‡ (kg ha ⁻¹)	89.6-112.0		89.6-112.0	
P Recommendation (kg ha ⁻¹)	56.0		56.0	
K Recommendation (kg ha ⁻¹)	145.6		145.6	

†Represents soil texture from equal parts silt loam, sand, and organic matter.

‡ N, P, and K recommendations are based for field grown production sugarcane along with the nutrient status.

subject. The interpretation of the chemical analysis is based on normal production sugarcane rather than reproductive sugarcane breeding genotypes. Because potting media differ greatly from agricultural soils in their physical and chemical properties, the interpretations in Table 2 may not apply. Growing plants in pots require different management practices than growing plants in the field. The nutrient concentrations in the soil may have been adequate for proper plant growth with a proper soil pH. Further nutrient evaluation of the potting media was conducted based on leaf tissue samples from sugarcane breeding genotypes that were grown in the potting media.

Results from Chapter 3 suggested that leaf nitrogen content prior to the artificial photoperiod regime varied due to pre-photoperiod fertilizer treatments. The leaf nitrogen content

of the sugarcane genotypes ranged from 12.4 g kg⁻¹ (Nitrogen treatment) to 9.7 g kg⁻¹ (No-nitrogen treatment). According to the leaf nutritional status of the crop that was developed by Anderson and Bowen (1990), these nitrogen concentrations were classified as low to very low, respectively. Although A&L Great Lakes Laboratories (Anonymous 2007b) report that potting media >200 mg kg⁻¹ of available nitrogen is high, the results in Table 2 support past findings that excessive watering is detrimental to nitrogen uptake.

This study concentrated on reproductive sugarcane genotypes. A high carbon to nitrogen ratio is desired for vegetative sugarcane entering the reproductive phase of growth (Sylvia et al. 1998). This was accomplished by eliminating nitrogen fertilization (Trt 1) several weeks prior to the beginning of the artificial photoperiod regime. The leaf potassium content for the combined genotypes used in this study ranged from 13.7 g kg⁻¹ (Nitrogen fertilizer) to 13.4 g kg⁻¹ (No-nitrogen fertilizer) which was considered to be in the optimum nutrient level for field production sugarcane at approximately the same age (Anderson and Bowen 1990). This adequate potassium leaf nutrient content contradicts the low chemical soil analysis for potassium that was taken at the beginning of the study. The leaf nutrient content also suggest that phosphorus was greater than the optimum nutrient level for field production sugarcane at approximately the same age. The difference between the soil analysis and the leaf analysis suggested that the macronutrients were available throughout the growth of the sugarcane.

The added fertilizers for the sugarcane breeding genotypes appeared adequate in addition to the potassium soil level that was available in the potting media prior to the experiment. The other macronutrient levels were nutrient residuals from the potting media (Table 2). Low pH, such as that observed in the soil media, tend to bind phosphorus and potassium such that they can become unavailable to the plant (Ashman and Puri 2002). However, leaf tissue analysis (Chapter

3) tended to suggest that there was an adequate supply of phosphorus and potassium. One explanation may be the constant watering of the potted plants that is required since the potting media easily dries out. The quality of the water is important because an enormous amount of watering is needed to produce the sugarcane breeding genotypes in pots. Water quality has been known to affect a change in media pH in potted plants for greenhouse production because of constant irrigation. The neutral pH water (pH=7.0) at the LSU AgCenter's Sugar Research Station that was used for constant irrigation likely increased the pH of the media in the pot culture (Anonymous 2007a) making the nutrients available.

Vegetative Results

The vegetative traits, stalk number ($P=0.62$), stalk height ($P=0.99$), and stalk diameter ($P=0.44$), were shown to have no significant genotype*nitrogen interaction (Table 3). The vegetative traits with a highly significant nitrogen effect were stalk number ($P\leq 0.01$), stalk height ($P\leq 0.01$), and stalk diameter ($P\leq 0.01$). Thus, it appears that nitrogen supplied to the sugarcane breeding genotypes prior to an artificial photoperiod regime (Trt 1-6) provided adequate vegetative growth prior to the reproductive phase change. Nitrogen significantly increased stalk number for all varieties, which is vital for adequate tasseling in sugarcane. The pre-photoperiod nitrogen treatments also provided a significant increase in both stalk height and stalk diameter compared to the pre-photoperiod no-nitrogen treatments. Stalk height is important because it is necessary for sugarcane to have 2-4 internodes when changing from the juvenile stage to the adult vegetative stage in preparation for the initiation stage (Burr et al. 1957). Stalk diameter was equally important because it provided for healthy stalks that are needed to support the sugarcane tassels. An adequate amount of stalks with adequate height and diameter provide a

nutritionally healthier plant for the reproductive stages. An adequate amount of stalks is important to sugarcane breeders who desire enough tassels for sugarcane hybridization.

Reproductive Results

Because nitrogen was important for the vegetative growth stage of the sugarcane breeding genotypes, further analysis were done to define the optimum post-photoperiod fertilizer treatment. Because total tassel number was dependent upon total stalk number, total tassel number was also significantly increased by the pre-photoperiod nitrogen fertilizer treatments. Orthogonal contrasts indicated that nitrogen significantly affected total stalk number in which the pre-photoperiod nitrogen treatments (Treatments 1-6) were found to be superior (Table 4). Stalk number was shown to have a highly significant genotype*nitrogen effect ($P \leq 0.01$). Because of the significant interaction effect, each genotype was analyzed separately. The results showed

Table 3. Treatment means for vegetative response variables. Experiments conducted at St. Gabriel, Louisiana during 2003 and 2004.

Treatment Number	Df	Pre-Photoperiod Treatments	Stalk Number	Stalk Height	Stalk Diameter
1		22.4-22.4-22.4	6 ab†	120 a	21 a
2		22.4-22.4-22.4	6 ab	118 a	21 a
3		22.4-22.4-22.4	6 ab	121 a	21 a
4		22.4-22.4-22.4	6 ab	131 a	21 a
5		22.4-22.4-22.4	6 ab	128 a	21 a
6		22.4-22.4-22.4	6 a	123 a	21 a
7		0-22.4-22.4	5 c	67 c	15 c
8		0-22.4-22.4	5 c	78 bc	16 bc
9		0-22.4-22.4	5 c	85 b	17 b
10		0-22.4-22.4	5 bc	73 bc	15 c
11		0-22.4-22.4	5 c	78 bc	15 bc
12		0-22.4-22.4	5 abc	70 bc	15 bc
Test of Fixed Effects	-----Probability values-----				
Trt	11		<.01	<.01	<.01
Genotype	2		<.01	<.01	.02
Trt*Genotype	22		.62	.99	.44

† Letter groupings were converted using the PDMIX800 macro in SAS (Saxton 1998).

that LCP85-384 tillered more profusely than HoCP85-845 and LCP86-454. Since the pre-photoperiod no-nitrogen treatments proved inferior and impractical due to a lack of stalks, only post-photoperiod treatments (1-6) were analyzed. Post-photoperiod nitrogen treatment effects on tasseling were determined by orthogonal contrasts with and without levels of nitrogen (Table 5). Specific orthogonal contrasts of interest were a no nitrogen fertilizer treatment contrasted to a high nitrogen fertilizer treatment. Other orthogonal contrasts of interest were the current LSU fertilizer rate (5.6-22.4-22.4 kg ha⁻¹) contrasted to a higher nitrogen fertilizer rate (44.8-22.4-22.4 kg ha⁻¹). Nitrogen did not significantly increase tassel number. There were no nitrogen by genotype interactions nor were there any significant nitrogen effects. The data analysis showed

Table 4. Treatment means, analysis of variance, and orthogonal contrasts for the pre-photoperiod nitrogen treatments that did result in a significant Treatment by Genotype interaction.

Treatment Number	df	Pre-Photoperiod Treatments	Post-Photoperiod Treatments	Stalk Number	Genotype		
					LCP85-384	LCP86-454	HoCP85-845
1		22.4-22.4-22.4	0-22.4-22.4	4	5 a	2 cde	4 bc
2		22.4-22.4-22.4	2.8-22.4-22.4	5	6 a	3 bcd	5 a
3		22.4-22.4-22.4	5.6-22.4-22.4	4	5 a	3 abc	4 abc
4		22.4-22.4-22.4	11.2-22.4-22.4	5	5 ab	4 ab	5 a
5		22.4-22.4-22.4	22.4-22.4-22.4	3	4 b	3 abc	3 c
6		22.4-22.4-22.4	44.8-22.4-22.4	5	6 a	4 a	5 ab
7		0-22.4-22.4	0-22.4-22.4	2	2 c	1 ef	2 d
8		0-22.4-22.4	2.8-22.4-22.4	1	2 cd	2 def	1 def
9		0-22.4-22.4	5.6-22.4-22.4	2	1 cd	1 ef	2 de
10		0-22.4-22.4	11.2-22.4-22.4	1	1 ce	1 ef	1 ef
11		0-22.4-22.4	11.2-22.4-22.4	1	1 ce	1 ef	1 ef
12		0-22.4-22.4	44.8-22.4-22.4
Test of Fixed Effects	-----Probability values-----						
Trt	11			<.01	†<.01	<.01	<.01
Genotype	2			<.01	.	.	.
Trt*Genotype	22			<.01	.	.	.
Contrast Probability							
Nitrogen Response							
Trt. 1-6 vs.Trt.7-12	1			<.01	.	.	.

† Mean separation was produced by PDMIX800 in SAS which is a macro for converting mean separation output to letter groupings (Saxton 1998).

that after the sugarcane breeding genotypes had switched from vegetative growth to reproductive growth, neither a lack of nitrogen nor the excessive nitrogen had any effect on sugarcane tasseling.

For the post-photoperiod fertilizer treatments (Table 5), inflorescence length and peduncle diameter (Trt*Genotype not significant) were the only reproductive variables significantly affected by the nitrogen treatments. Orthogonal contrasts indicated that the no-nitrogen treatment (Trt 1) significantly decreased inflorescence length. Treatment 2 (2.8-22.4-22.4 kg ha⁻¹) also significantly decreased inflorescence lengths while treatments 3, 4, 5, and 6 significantly increased inflorescence lengths. An increase in inflorescence length could be a result of two causes. First, the inflorescence main axis was elongated with the same number of

Table 5. Treatment means, analysis of variance, and orthogonal contrasts for the post-photoperiod nitrogen treatments that did not result in a significant Treatment by Genotype interaction.

Treatment Number	df	Post-Photoperiod Treatments	Tassel Number	Inflorescence Length (cm)	Mean Flag Leaf Duration (Days)	Mean Flag Leaf Date†	Mean Anthesis Date‡	Mean Peduncle Diameter (mm)
1		0-22.4-22.4	4 bc	59 b	26 a	250 a	275 a	5.2 e
2		2.8-22.4-22.4	4 ab	61 b	24 ab	247 a	274 a	5.8 d
3		5.6-22.4-22.4	4 abc	66 a	24 ab	248 a	273 ab	6.4 c
4		11.2-22.4-22.4	5 ab	66 a	23 b	245 a	267 b	6.9 b
5		22.4-22.4-22.4	4 c	67 a	23 b	248 a	271 ab	7.3 a
6		44.8-22.4-22.4	5 a	67 a	23 b	248 a	269 ab	7.3 a
Test of Fixed Effects	-----Probability values-----							
Trt	5		.05	<.01	.15	.74	.12	<.01
Genotype	2		<.01	<.01	.01	<.01	<.01	<.01
Trt*Genotype	10		.30	.25	.66	.34	.96	.89
Contrast Probability								
Nitrogen Response								
Trt. 1 vs. Trt. 6	1		.04	<.01	.03	.44	.13	<.01
Trt. 3 vs. Trt. 6	1		.45	.24	.35	.98	.07	<.01

†, ‡ Mean flag leaf date, Mean inflorescence date is represented in Julien Date.

lateral axis. Secondly, the main axis was elongated with additional lateral axes which would provide more flowers per inflorescence. More flowers per inflorescence have the ability to increase viable seed production.

Orthogonal contrasts indicated that nitrogen significantly increased mean peduncle diameter with regards to different fertilizer formulations. As nitrogen rates increased so did peduncle size for the six treatments analyzed. Although peduncle size had no direct relation to seed production, it is the supporting stem for the inflorescence and important during crossing as peduncles that are too slender tend to break. The remaining reproductive traits including tassel number ($P=0.06$), flag leaf duration ($P=0.15$), flag leaf date ($P=0.74$), and anthesis date ($P=0.12$) had no significant nitrogen effects.

SUMMARY

Because of Louisiana's temperate climate, the LSU AgCenter's Sugarcane Breeding Program relies on artificial photoperiod regimes to induce tasseling. Sugarcane breeders have limited information about sugarcane nutrition as a means of maximizing tasseling. This research was performed to determine the impact of nitrogen fertilization prior to and during the time of induction for sugarcane breeding genotypes grown in pots and to determine if the current nitrogen fertilizer treatments were appropriate. Apart from recording tassel number, data were also collected during different stages of sugarcane growth on agronomic (stalk height, stalk number, and stalk diameter) and reproductive (inflorescence length, flag leaf duration, flag leaf date, anthesis date and peduncle diameter) traits.

The results reiterated the importance of sufficient nitrogen prior to the artificial photoperiod regime. Sufficient nitrogen for this study consisted of a liquid nitrogen fertilizer ($22.4-22.4-22.4 \text{ kg ha}^{-1}$) in addition to a potting media with a high amount of available nitrogen.

Tassel number was higher among the group of plants that received nitrogen fertilizer prior to the artificial photoperiod regime compared to the group that received a no-nitrogen fertilizer. A wide array of nitrogen levels were eventually applied to both groups of plants as post-photoperiod treatments in which none could compensate for the no-nitrogen pre-photoperiod treatment. Apparently, plants that received nitrogen fertilizer prior to the artificial photoperiod regime were healthier and better prepared for the transition from the vegetative to the reproductive stage. These plants were more numerous, taller, and thicker in diameter compared to those in the no-nitrogen group. Stalk number seems to be the most critical agronomic trait since treatments resulting in low stalk numbers are impractical and undesirable for the LSU AgCenter's Sugarcane Breeding Program. For this reason, only post-photoperiod treatments with pre-photoperiod nitrogen (Trts 1-6) were considered. For the reproductive traits, increasing nitrogen rates during the post-photoperiod treatment significantly increased inflorescence length ($P \leq 0.01$) and peduncle diameter ($P \leq 0.01$). Tasseling along with the other reproductive traits did not seem to respond to varying rates of nitrogen applied during the post-photoperiod treatment.

All aspects of nitrogen availability must be evaluated for any sugarcane breeding program that uses pot culture for its breeding genotypes. Nitrogen availability can be a result of factors which include nitrogen composition of the potting media as well as supplemental nitrogen. Other nutrients can be affected by the pH of the potting media in addition to the pH of the irrigation water. For the potting media that was used in this study, the LSU fertilizer rates consisting of a 22.4-22.4-22.4 kg ha⁻¹ pre-photoperiod treatment followed by a 5.6-22.4-22.4 kg ha⁻¹ post-photoperiod treatment cannot be discounted as being optimum. Further exploration of multiple nitrogen rates prior to the artificial photoperiod regime (agronomic traits) may uncover significant findings in relation to sugarcane tasseling. Additional research of multiple nitrogen

rates on inflorescence length (reproductive trait) may uncover significant findings in relation to sugarcane seed production.

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CHAPTER 3
THE EFFECT OF NITROGEN ON LEAF MACRONUTRIENT LEVELS
OF SUGARCANE BREEDING GENOTYPES IN POT CULTURE

INTRODUCTION

At the LSU AgCenter's Sugar Research Station, artificial photoperiod regimes are necessary to achieve sugarcane tasseling due to Louisiana's cool fall temperatures that occur during the typical initiation phase of sugarcane reproduction. The LSU AgCenter's Sugarcane Breeding Program makes use of artificial photoperiod regimes to move the initiation phase to a more conducive reproductive environment. The age of the sugarcane breeding genotypes undergoing artificial photoperiod regimes is shortened by two to three months due to the impending cold weather. Sugarcane breeding genotypes are subjected to artificial photoperiod regimes in pot culture which are placed on rail carts. Nitrogen, an essential nutrient for crop growth and development is applied to sugarcane in pot culture. There is scarcity of information on how nitrogen affects sugarcane growth in pot culture, let alone the relationship between nitrogen and other macronutrients in this growing media. A better understanding of this relationship could eventually lead to new management practices to improve tasseling among sugarcane breeding genotypes.

Plant nutrient analysis has been used to make fertilizer evaluations and to recommend corrections for nutrient deficiencies (Smith and Loneragen 1997). Nutrient requirements change throughout the growth and development of a plant. Nutrient levels at certain stages of growth influence the yield of the economically important tissues (Taiz and Zeiger 2002). Proper use of plant tissue analysis requires an understanding of the relationship between plant growth and the mineral concentration of plant tissue samples (Bouma 1983). Sugarcane plant analysis for crop

production has been extensively researched, whereas little research has been done on sugarcane plant analysis for reproductive purposes.

The levels of nitrogen, phosphorus, and potassium within a plant can affect the growth and transformation of the plant from the vegetative to reproductive stage. Nitrogen, for example, is known to be a major factor limiting grass seed production (Loch et al. 1999). Since one of nitrogen's functions is to dictate the amount of chlorophyll that a plant contains (Reuter and Robinson 1997), a chlorophyll meter was used in this study to collect chlorophyll readings from the same leaves that were sampled for plant analysis. If nitrogen was found to be a major controlling nutrient in the tasseling of sugarcane breeding genotypes, and chlorophyll and nitrogen levels were found to be positively associated, then chlorophyll monitoring might be useful in indirectly detecting nitrogen levels during the progression of sugarcane tasseling. Chlorophyll monitoring is less expensive and less time consuming than the typical nitrogen leaf sample analysis that has traditionally been used to monitor crop growth.

The objectives of this study were: (i) to determine the effect of nitrogen on other macronutrients in sugarcane at various stages of the sugarcane reproduction cycle and (ii) to determine the relationship between chlorophyll and nitrogen levels at various stages of the sugarcane reproduction cycle. The plants sampled (leaf samples) in this study were grown in pot culture and subjected to artificial photoperiod regime.

MATERIALS AND METHODS

Design of Experiment

A two-year (2003-2004) plant nutrition experiment was conducted with three sugarcane genotypes used for hybridization at St. Gabriel, Louisiana (latitude 30° 15' N, longitude 91° 05' W). The three genotypes were LCP85-384 (Milligan et al. 1994), LCP86-454 (Martin et al.

1996), and HoCP85-845 (Legendre et al. 1994). All three genotypes possess qualities of high genetic value that make each of them important for breeding. All three sugarcane genotypes are classified as “easy-to-induce” (LaBorde et al. 2004).

The sugarcane genotypes used in this experiment were vegetatively propagated during the months of October 2002 and October 2003 for the subsequent breeding seasons. Eye pieces were planted into styrofoam trays that contained Metro Mix 350 horticultural mix (Sun Gro, Canada) with a supplement of 3 g Peter’s fritted trace elements, 453.60 g dolomitic limestone, and 907.2 g of superphosphate (Martin 1994). The genotypes were fertilized according to the recommendations of the LSU AgCenter’s Sugarcane Breeding Program (LaBorde et al. 2004). During January 2003 and January 2004, the sugarcane breeding genotypes were transplanted into 37.8 L pot culture. The soil media consisted of equal parts of washed sand, Canadian peat moss (Sun Gro, Canada), and a Commerce silt loam (fine-silty, mixed, nonacid, thermic aeric Fluvaquents) soil. The Canadian peat moss improved moisture-holding capacity, the relationship of air to water, and the ability to physically manage the media. Both sand and Canadian peat moss creates and enhances a very desirable plant growing media (Donahue et al. 1983) with the addition of commerce silt loam. The major chemical effect of soil organic matter (SOM) in most soils is that it contributes 20% to 80% of the cation exchange capacity (CEC) that is proportionate to the soil pH (Sylvia et al. 1998).

Genotypes were maintained in pot culture for the remainder of the experiment. Each experimental unit consisted of one 37.8 L pot with a surface diameter of 40.6 cm and a height of 36.8 cm. Each experimental unit contained two sugarcane setts that were allowed to tiller. Once two tillers were established for each primary stalk, all excess tillers were deliberately removed from each pot for a maximum of six potential sugarcane stalks. Tillering would be desirable to

attain an equal number of stalks in each pot, however, many pots with specified treatments failed to produce an adequate number of tillers.

The experimental design for each year was a completely randomized design that included 12 fertilizer treatments in a two-factor factorial treatment arrangement. Factor one consisted of pre-photoperiod fertilizer treatments whereas factor two consisted of post-photoperiod fertilizer treatments. Pre-photoperiod and post-photoperiod fertilizer treatments coincided with the vegetative and reproductive phases, respectively. Only the pre-photoperiod nitrogen fertilizer was deemed to be significant in optimizing sugarcane tasseling (Chapter 2). The sampling units for each experimental unit were sugarcane leaves.

Fertilizer Treatments

After the sugarcane breeding genotypes were transplanted into large pot culture, the fertilizer treatments were applied on a biweekly basis. During the pre-photoperiod treatments, the two different fertilizer treatments consisted of a nitrogen (Trt 1) and a no-nitrogen treatment (Trt 2). The nitrogen treatment consisted of a liquid formulation of Peters 22.4-22.4-22.4 kg ha⁻¹ whereas the no-nitrogen treatment consisted of a granular fertilizer formulation of 0-22.4-22.4 kg ha⁻¹. The pre-photoperiod fertilizer treatments began in January of each year and ended three weeks prior to the beginning of the artificial photoperiod regimes on May 30. Fertilizer treatments ceased in May in order to raise the carbon nitrogen ratio in the soil which is desirable for promoting sugarcane tasseling (Martin 1994). The liquid formulation was applied at a rate of 3.5 L per 37.8 L pot. The granular formulation was applied at a rate of 29.6 ml per 37.8 L pot. These two treatments were aimed to enhance (nitrogen) and stress (no- nitrogen) the physiological processes needed for sugarcane reproduction.

The liquid nitrogen fertilizer was applied through the watering system with the aid of a Dosatron fertilizer injector (Dosatron, Clearwater, FL). When connected to the watering system, the Dosatron injector mixed stock solution into the water at a ratio of 1:100, which produced a watering solution that contains 400 mg kg⁻¹ each of N-P-K. The granular fertilizer formulations were thoroughly mixed by hand in a tub. The sources of phosphorus and potassium for the granular formulation were triple superphosphate (46%) P₂O₅, and potash (60%) K₂O. Vermiculite was used as filler in order to calibrate correct formulation for some of the treatments.

Data Collection

Leaf Sampling

Nutrient analysis was done on leaves of sugarcane breeding genotypes grown in pot culture at four different growth stages (Fig. 2). The leaf sampling technique for this study included the first leaf below the top visible dewlap (Anderson and Bowen 1990) for all growth stages except the anthesis stage. At the anthesis stage, the top visible dewlap no longer existed because of morphological differences between vegetative and reproductive sugarcane. For this reason, the flag leaf was sampled for nutrient analysis. Although researchers have established differentiated nutrient levels for leaves with and without the midrib (Muchovej et al. 2005), this study included the midrib because it was more convenient for sampling purposes. For the vegetative and initiation stages, sampling was done for all stalks in each experimental unit because it was too early to distinguish between the physiological stages. For the elongation and anthesis stages, sampling was done for individual stalks that showed signs of entering the reproductive stage. Leaf sampling for the elongation stage was done on the same stalks at a subsequent stage, the anthesis stage.

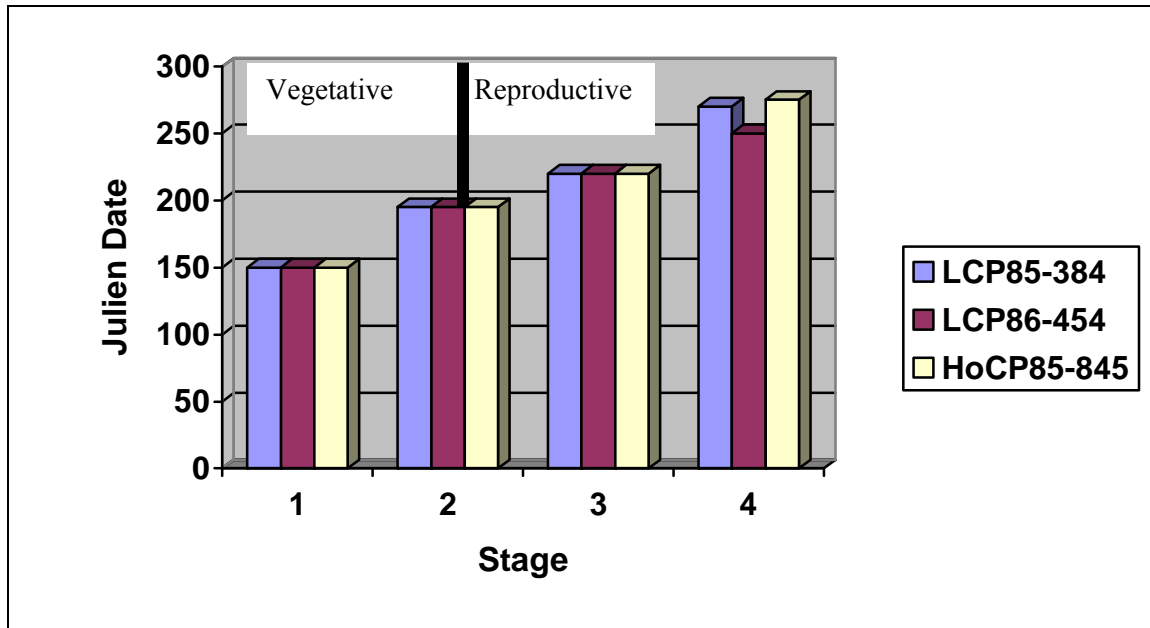


Fig. 2. Visual of different stages of the tasseling developmental pathway and three genotypes. Stage 1 is the vegetative stage, stage 2 is the initiation stage, stage 3 is the elongation stage, and stage 4 (†) is the anthesis stage. The line on stage 2 signifies that it is a transition stage between vegetative growth and reproductive growth.

† Stage 4 consists of differences in Julian date because each genotype tasseled at a different average date.

The three sugarcane genotypes were sampled five times each for every stage of the pre-photoperiod nitrogen and the pre-photoperiod no-nitrogen fertilizer treatments. This sampling was done in each of two years. The sequence of progression in the tasseling of sugarcane consisted of a normal developmental pathway (tasseling) or an alternative developmental pathway (nontasseling / vegetative reversion). Moore and Nuss (1987) considered the interruption in the normal sequence of development to be the alternative pathway or alternative morphology. The sampling stages represent four different stages (Figure 2) of sugarcane genotype growth for reproductive purposes (Moore and Nuss 1987). The first stage of the reproductive life cycle was prior to the artificial photoperiod regime and was referred to as the vegetative stage; the second stage was equivalent to the apical meristem transitioning from the

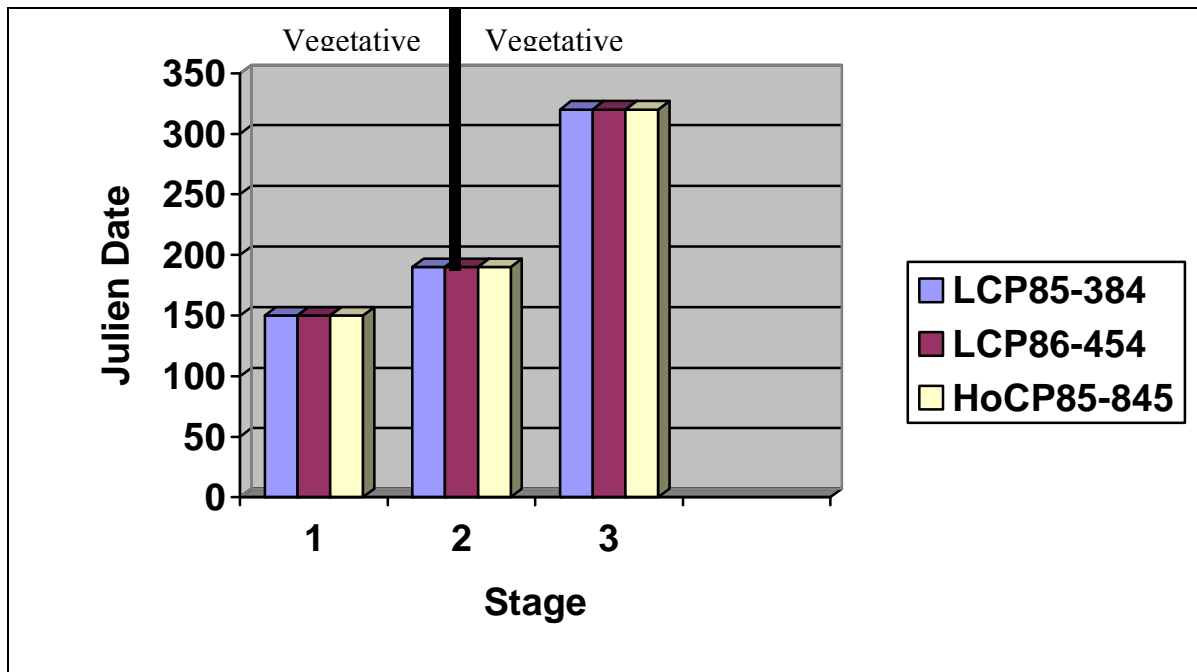


Fig. 3. Visual of different stages of nontasseling developmental pathway and three genotypes. Stage 1 is a vegetative stage, stage 2 is the initiation stage, and stage 3 is a vegetative stage due to a lack of a reproductive transition point that is represented by the line on stage 2.

vegetative phase of growth to the reproductive phase of growth and was referred to as the initiation phase; the third stage was the first visible sign of induction and was referred to as the elongation stage; and the fourth stage was referred to as the anthesis stage. The sugarcane nonreproductive or vegetative life cycle is represented by the different stages in Figure 3. After stage 2, nutrient levels were obtained only from leaf samples that eventually entered the reproductive pathway (Figure 2).

Nutrient Analysis

The same leaf analysis was performed for each of the stages except for Stage 4, the anthesis stage. Because of the reduced size of a normal flag leaf during anthesis, there was only enough leaf tissue (0.1 g) to run a nitrogen analysis. The additional 0.5 g needed for an additional macronutrient analysis was not available. Figure 4 describes the sampling arrangement that was used for this study. The leaf analysis was performed at the LSU

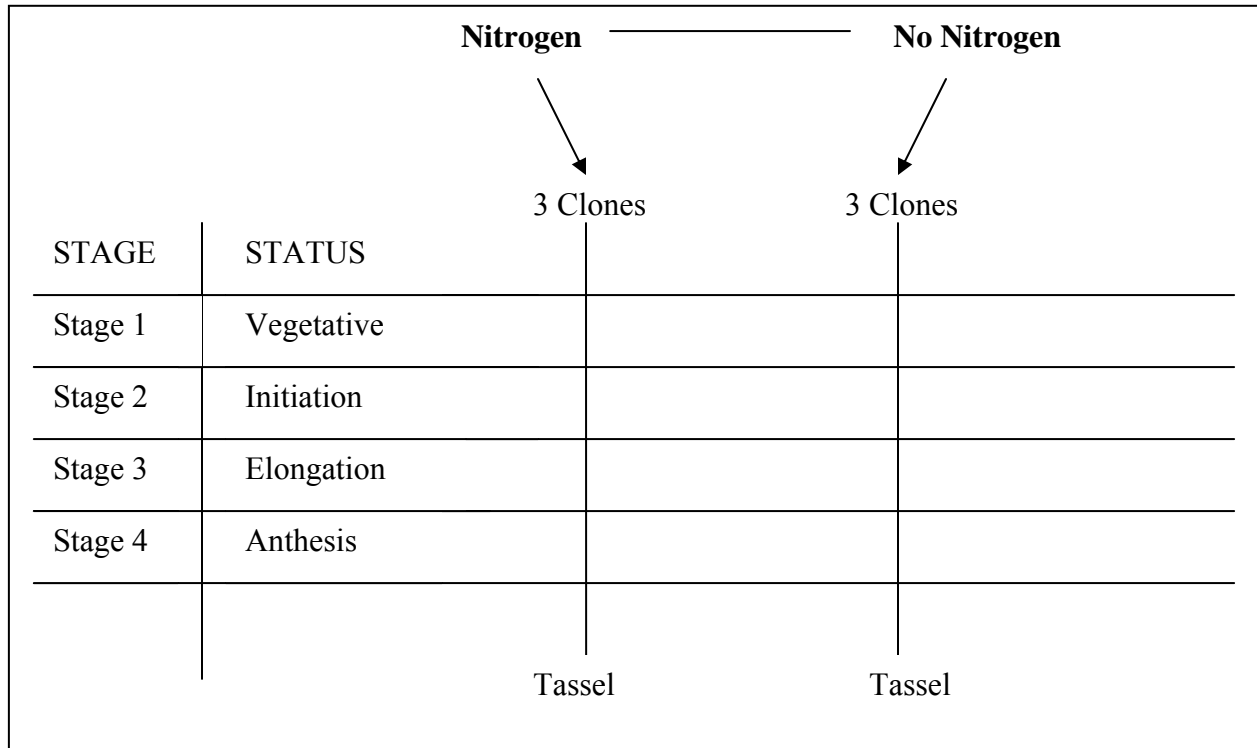


Fig. 4. Visual of the nutrient analysis experimental design. Treatments consisted of a nitrogen versus a no-nitrogen for the reproductive developmental pathway at different stages of growth.

† Stage is represented in Figure 2 for the tasseling developmental pathway.

AgCenter’s Soil Testing and Plant Analysis Laboratory (STPAL); an AgRoutine metals package and nitrogen test were done for all leaf sampling stages except for stage four. The macronutrients of interest determined by the metals package consisted of P, K, Ca, Mg, and S. The procedure used for the analysis of AgRoutine metals is as follows: a 0.5 g leaf tissue sample is mixed with 5 ml concentrated HNO₃ for 50 min, then 3 ml H₂O₂ is added, digested for 2.75 h on a heat block, the mixture is cooled, diluted and read on ICP (Spectro Ciros, Fitchburg, MA). The procedure used for nitrogen analysis (Anonymous 2005) consisted of a 0.1 g leaf tissue sample that is analyzed by dry combustion of a Leco N analyzer. The results of the leaf tissue analysis were based on samples that were analyzed with the leaf midrib.

Macronutrient Reference Points

Although all sugarcane in this study was grown in pot culture for the purpose of reproduction, the only available data to use as a reference point for comparison were derived from field grown sugarcane (Anderson and Bowen 1990) and are shown in Table 6. These levels represent the standard leaf-nutrient concentrations for vegetatively/commercially grown sugarcane in Louisiana. The results represent leaf nutrient concentrations for field grown sugarcane (three months of age) that is supposedly equivalent to the same growth stage as the sugarcane in the vegetative stage prior to the artificial photoperiod regimes. The table also provides critical nutrient levels along with the optimum nutrient levels for several macronutrients for field grown sugarcane although all sugarcane in this study was in pot culture. For all macronutrients in the vegetative stage in Table 7, only nitrogen and sulfur fall below the critical nutrient levels listed in Table 6. Because critical nutrient levels have not been developed for the

Table 6. Critical and optimum nutrient levels for various macronutrients of plant tissue analysis for Louisiana sugarcane grown in the field.†

Nutrient	Units	Critical Nutrient Level	Optimum Nutrient Level	Tissue Age (mo.)
Nitrogen	g kg ⁻¹ dry weight	12.5	15.0-17.5	3-4
Phosphorous	g kg ⁻¹ concentration	1.4	1.8-2.2	3
Potassium	g kg ⁻¹ concentration	10.0	12.5-17.5	3
Sulfur	g kg ⁻¹ concentration	1.3	1.3-1.8	3-4
Calcium	g kg ⁻¹ dry weight	1.5	2.8-4.7	3-4
Magnesium	g kg ⁻¹ dry weight	0.8	1.4-3.3	3-4

†Midrib included in the leaf tissue analysis. The results are based on the Top Visible Dewlap (From Anderson and Bowen 1990).

purpose of sugarcane reproduction in pot culture, the comparison of macronutrient levels in Table 6 was restricted to only the vegetative stage of this experiment.

Chlorophyll Readings

A lightweight, portable instrument developed by the Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Company (Spectrum Technologies, Plainfield, IL) was used to estimate chlorophyll levels in the sugarcane leaves. The SPAD meter estimated the amount of chlorophyll by measuring the amount of light transmitted through the leaf (Stevens and Hefner 2005). After recording average meter readings from the bulk field and reference area, a nitrogen sufficiency index (Anonymous 2006) can be calculated by multiplying by 100. The SPAD meter was used to estimate chlorophyll levels in the same leaves that were sampled in 2003-2004 for nutrient analysis with the exception of the flag leaf at the anthesis stage. Due to the small size of the flag leaf at the anthesis stage, the chlorophyll reading was taken on the leaf immediately below the flag leaf. Due to slight variations in chlorophyll content that can occur within a leaf (Bonneville and Fyles 2006), the chlorophyll level of each leaf was sampled four times and averaged. The chlorophyll content of each leaf was measured approximately midway the length of the leaf. The portion of the leaf between the midrib and the leaf margin was measured because this portion of the leaf was the only portion that could be measured by the SPAD meter as it is unable to close completely over the midrib.

Statistical Analysis

Least square means values of nutrient levels within each stage for the nitrogen and non-nitrogen treatment were computed in SAS. Comparison between pairs of means was achieved using the single degree of freedom contrast approach (Schlotzhauer and Littell 1997). Correlation coefficients determined the linear relationship between percent tasseling and leaf

macronutrient levels. Correlation coefficients estimated the strength of the linear relationship between leaf nitrogen content and chlorophyll levels. Correlation coefficients were computed with the Proc Corr procedure in SAS (Schlotzhauer and Littell 1997). The Proc Mixed with LS Means Statement procedure in SAS (Schlotzhauer and Littell 1997) was used to report the leaf macronutrient level and the chlorophyll levels in the sugarcane leaves. The nutrients were segregated for both pre-photoperiod nitrogen and pre-photoperiod no-nitrogen fertilizer treatments to assess the importance of all nutrients for tasseling.

RESULTS AND DISCUSSION

Nitrogen has been known to affect the movement of other nutrients from older leaves to younger leaves where the location of the deficient nutrient symptoms may vary with the level of nitrogen in the plant (Reuter and Robinson 1997). The effect of nitrogen on macronutrient levels in this study was based upon a nitrogen (Trt 1) and no-nitrogen (Trt 2) fertilizer treatment containing equal amounts of phosphorous and potassium. However, the degree to which the data can be affected by our inability to distinguish nutrients obtained from fertilization from nutrients that were already present in the inorganic soil or plants prior to the experiment is unknown. Also, soil pH (e.g. low pH) can affect nutrient availability to the plant irrespective of whether nutrients are present in the soil. In this study, soil testing prior to the experiment revealed low soil pH (4.77 in 2003; 4.63 in 2004) conditions. However, constant irrigation with neutral pH water may have raised (Anonymous 2003) the pH levels enough to enable plant nutrient availability.

Sugarcane that received the nitrogen treatment tasseled profusely (77%), whereas the no-nitrogen treated sugarcane tasseled poorly (25%). Most of the macronutrient levels for the no-nitrogen treatment were consistently higher than those for the nitrogen treatment (Table 7). It is

likely that because the no-nitrogen treatment had a significantly lower number of stalks than the nitrogen treatment (Chapter 2), this resulted in a higher nutrient uptake on a per plant basis.

Table 7. Mean leaf nutrient levels (\pm standard error) for macronutrients in the tasseling and nontasseling developmental pathways for the nitrogen (1) and no-nitrogen (2) treatment of sugarcane plants grown in pot culture.

Nutrient	Treatment	Stage§ 1	Stage 2	Stage† 3	Stage‡ 4
Nitrogen (g kg ⁻¹ dry weight)	1	12.4 \pm .03	11.5 \pm .04	12.8 \pm .06	10.8 \pm .06
	2	9.7 \pm .03	14.1 \pm .05	14.9 \pm .06	12.2 \pm .07
	Comparison¥	*	*	*	*
Phosphorus (g kg ⁻¹ concentration)	1	2.3 \pm .01	1.9 \pm .01	2.0 \pm .01	.
	2	2.7 \pm .01	2.4 \pm .01	2.1 \pm .01	.
	Comparison	*	*	ns	.
Potassium (g kg ⁻¹ concentration)	1	13.7 \pm .04	11.1 \pm .02	11.0 \pm .03	.
	2	13.4 \pm .02	13.4 \pm .02	12.6 \pm .03	.
	Comparison	ns	*	ns	.
Sulfur (g kg ⁻¹ concentration)	1	0.9 \pm .00	0.8 \pm .00	0.9 \pm .00	.
	2	1.0 \pm .00	1.1 \pm .00	1.1 \pm .00	.
	Comparison	*	*	*	.
Calcium (g kg ⁻¹ dry weight)	1	2.5 \pm .01	1.9 \pm .01	2.0 \pm .01	.
	2	2.4 \pm .01	2.1 \pm .01	2.2 \pm .01	.
	Comparison	ns	ns	ns	.
Magnesium (g kg ⁻¹ dry weight)	1	1.1 \pm .11	0.9 \pm .00	0.9 \pm .00	.
	2	0.9 \pm .00	0.9 \pm .00	0.9 \pm .00	.
	Comparison	ns	ns	ns	.

† Stage 3 represents sugarcane in the tasseling developmental pathway.

‡ Stage 4 (flag leaf) consisted of only enough material to perform a nitrogen analysis.

§ Stage 1, 2, 3, and 4 correspond to the vegetative, initiation, elongation, and anthesis stages, respectively.

¥ Comparison among pairs of means values within a stage was made using single degree of freedom contrasts.

*, ns Significant and not significant at the 0.05 probability level, respectively.

Comparable studies looking at leaf nutrient levels in reproductive sugarcane are not available for referencing with the results of this study. Critical leaf nutrient levels for three month old field grown sugarcane (Anderson and Bowen 1990; Table 6) are the best available data that coincided with the vegetative (Stage 1) stage of this study. All comparisons to previous critical leaf nutrient levels in this study are restricted to Stage 1.

Primary Macronutrients

Nitrogen

For the vegetative stage, the leaf nutrient level for the nitrogen treatment was 12.4 g kg^{-1} while the leaf nutrient level for the no-nitrogen treatment was 9.7 g kg^{-1} (Table 7). Leaf nitrogen levels for both treatments were below the critical nutrient levels listed on Table 6. Since tasseling in the nitrogen and no-nitrogen treatments were 77% and 25%, respectively, the critical leaf nutrient level at the vegetative stage for sugarcane intended for reproductive purposes should be around 12.4 g kg^{-1} . Nitrogen promotes early vegetative growth (Gosnell 1973), which is critical in the shoot apex changing from the juvenile phase to the adult vegetative phase. In sugarcane, this phase change is generally associated with the development of two to four internodes at the base of the stalk (Burr et al. 1957). Because nitrogen serves as a constituent of many plant cell components, such as amino acids and nucleic acids, nitrogen deficiency can rapidly inhibit plant growth (Taiz and Zeiger 2002). Therefore, the increased level of leaf nitrogen for the nitrogen treatment provided for a healthy plant prior to the plants transition to the reproductive phase.

However, although nitrogen is important in the initial vegetative growth, excess nitrogen may actually be detrimental to tasseling as it may cause the plants to remain in the vegetative growth phase. A high carbon to nitrogen ratio in the soil and plant is desirable for promoting

Table 8. Correlation coefficients for the two nitrogen treatments (nitrogen, 1; no-nitrogen, 2) between leaf macronutrient levels and percent tasseling.

Nutrient	Treat- ment	Stage§ 1	Stage 2	Stage 3	Stage† 4
Nitrogen	1	0.15	-0.34*	-0.27	-0.25
	2	0.21	-0.28	-0.11	-0.19
Phosphorus	1	0.13	-0.24	-0.25	.
	2	0.06	-0.23	-0.04	.
Potassium	1	0.18	-0.14	-0.11	.
	2	-0.06	-0.28*	-0.41*	.
Sulfur	1	0.28*	-0.23	-0.16	.
	2	-0.01	-0.27	-0.15	.
Calcium	1	0.22	-0.23	-0.33	.
	2	0.13	-0.14	-0.05	.
Magnesium	1	0.22	-0.32	-0.49*	.
	2	0.05	-0.15	-0.20	.

† Stage 4 (flag leaf) consisted of only enough material to derive a nitrogen analysis.

* Represents that the model is significant at $\alpha=0.05$.

§ Stage 1, 2, 3, and 4 correspond to the vegetative, initiation, elongation, and anthesis stages, respectively.

sugarcane tasseling (Martin 1994). In this study, fertilizer application was ceased three weeks before the start of the artificial photoperiod regime in order to simulate the high carbon to nitrogen ratio. It is, therefore, not surprising that most of the correlation coefficients between nitrogen and percent tasseling beyond Stage 1 had negative signs although the only significant correlation ($r = -0.34$) occurred during the initiation stage (Table 8). The mean leaf nutrient level for the nitrogen treatment at the initiation stage was 11.5 g kg^{-1} which was significantly ($P \leq 0.05$) lower than the no-nitrogen treatment at 14.1 g kg^{-1} (Table 7). Nitrogen levels in subsequent stages (Stages 3 and 4) were also significantly ($P < 0.05$) higher for the no-nitrogen compared to the nitrogen treatment. These findings, although in pot culture, support Van Dillewijns (1952) conclusion that high levels of nitrogen during initiation may reduce or delay natural tasseling.

Phosphorus

Nitrogen had a significant ($P < 0.05$) effect on leaf phosphorus content in the earlier but not the latter stages of growth (Table 7), although the contribution to this result stemming from the differences in stalk number cannot be discounted. For the vegetative stage, the leaf phosphorus level for the nitrogen treatment was 2.3 g kg^{-1} , whereas the leaf phosphorus level for the no-nitrogen treatment was 2.7 g kg^{-1} (Table 7). Leaf phosphorus levels for both treatments (Table 7) were above the optimum nutrient level (Table 6). Although phosphorus is notoriously difficult to keep in plant available form (Ashman and Puri 2002), it appears deficiency was not a problem in this study, at least in the vegetative stage. The constant levels of phosphorus provided to both the nitrogen and no-nitrogen treatments seem to provide the necessary phosphorus nutrition. Thus, although phosphorus was generally lower among the group of plants receiving nitrogen compared to the group receiving no-nitrogen this did not affect flowering in the former group. Although a previous report stated that the application of phosphate promotes tasseling (Van Dillewijn 1952), the results in this study could not establish an association between percent tasseling and leaf phosphorus content (Table 8). Phosphorus levels and those of other nutrients were constant in this experiment and so any association between these nutrients and tasseling would have to depend first on their interaction with nitrogen.

Potassium

Besides nitrogen, potassium requirements are the largest needed by plants (Marschner 1997). Although the vegetative stage (Stage 1) had no significant associations between percent tasseling and leaf potassium content (Table 8), the leaf nutrient levels for the nitrogen treatment (13.7 g kg^{-1}) and the no-nitrogen treatment (13.4 g kg^{-1}) were in the optimum nutrient level as compared to the point of reference in Table 6. The slightly higher but not significant ($P > 0.05$)

leaf nutrient level for the no-nitrogen treatment was partly a result of fewer stalks in the no-nitrogen treatment having a higher nutrient uptake on a per plant basis than in the nitrogen treatment. This seems to be a recurring relationship for all of the primary macronutrients. Leaf potassium nutrient levels decreased from the initiation stage to the elongation stage (Table 7) and this was accompanied by a significant, negative association (Table 8), albeit weak, with tasseling in the no-nitrogen treatment. As with nitrogen, progress towards tasseling in sugarcane may also be associated with decreased levels of potassium. Menshawi (1978) observed increased potassium levels in the sugarcane apex shortly before initiation followed by a decline after initiation which may suggest that potassium had a role in changing the shoot apical meristem from the vegetative phase to the reproductive phase. Also, excessively low potassium leaf levels have been correlated with a high proportion of sterile female flowers (Marschner 1997) supporting the fact that potassium plays some role in reproduction. BrunkHorst (2001) also reported similar results where high levels of potassium appeared to improve the number of viable seeds per tassel in sugarcane.

Secondary Macronutrients

Sulfur fell below the critical nutrient level depicted in Table 6. Sulfur deficiency can inhibit protein synthesis leading to chlorosis (Marschner 1997), which can occur in both mature and young leaves because sulfur is not easily mobilized. Furthermore, the distribution of sulfur in sulfur-deficient plants is known to be affected by the nitrogen supply indicating that the extent of remobilization and retranslocation of sulfur from older leaves depends on the level of nitrogen deficiency (Taiz and Zeiger 2002). Nitrogen had a significant ($P < 0.05$) effect on sulfur levels in the three stages examined and both nutrients seem to display a similar trend within and across the stages. Because nitrogen fertilization was stopped for several weeks by the beginning of the

artificial photoperiod regime, the less than optimum levels of leaf sulfur detected in this study may have been due to the reduced levels of nitrogen administered to the plants.

Sulphur had a significant but low positive association with percent tasseling at the vegetative stage ($r = 0.28$) for the plants that received nitrogen (Table 8). All other associations were negative and not significant. However, because sulfur levels and mobility in the plant are dependent upon the amount of nitrogen in the plant, nitrogen is the more limiting macronutrient known to affect tassling (Table 8) and leaf sulfur levels would inevitably assume a minor role.

Calcium leaf nutrient levels (Table 7) in all stages were above the critical nutrient level but below optimum nutrient levels (Tables 6). Calcium leaf levels did not fluctuate much throughout the different stages of the reproductive life cycle and appear sufficient enough to provide tassels. Nitrogen had no significant ($P > 0.05$) influence on the leaf calcium content and calcium had no significant association with tasseling for any of the different stages of the reproductive life cycles (Table 8). Therefore, calcium may be of minor importance in sugarcane tassling.

Leaf magnesium nutrient levels in the vegetative stage were above critical levels but below optimum levels (Table 6). Menshawi (1978) found that leaf magnesium levels in sugarcane increased in the apical meristem during the period preceding initiation which would explain the decrease in magnesium levels in this study for the nitrogen treatment between the vegetative and initiation stages. Although this nutrient was not in the optimum nutrient level range, magnesium levels in the vegetative stage seemed to have no negative effect on tasseling. After remaining constant subsequent to the vegetative stage, magnesium content had a significant negative effect on tasseling at the elongation stage ($r = -0.49$) in which the leaf magnesium level was 0.9 g kg^{-1} (Table 7). One possible explanation deals with the source-sink

relationship between the younger leaves and the floral meristem. The upper mature leaves on a plant usually provide photosynthates to the growing shoot tip which in this case was the floral meristem (Taiz and Zeiger 2002). Magnesium deficiency can cause a decrease in photosynthetic rate which in turn can cause an increase in the levels of carbohydrates in the young leaf that was sampled. In other instances, decreased photosynthetic rates have resulted in leaf senescence that was readily induced by high light in combination with a magnesium deficiency (Marschner 1997). Due to the low levels (Table 6) of magnesium at the elongation stage (Table 7), the excessive carbohydrates that are formed decreased the development of the floral meristem. However, the weak association between leaf magnesium content and percent tasseling adds credence to the fact that overall magnesium may play a minor role in sugarcane reproduction.

Chlorophyll Results

A significant positive association was found between chlorophyll and nitrogen levels in both the nitrogen and no-nitrogen treatments throughout all stages (Table 9). Furthermore, although not significant, the association between chlorophyll and tasseling in all stages was similar in trend to that between nitrogen and tasseling. The SPAD reading (34.53) of chlorophyll coincides with the significant negative associations of leaf nitrogen and tasseling at the initiation stage (Table 8). The corresponding correlation coefficient between nitrogen and chlorophyll at this stage was high ($r = 0.80$) and significant (Tables 8 and 9). It appears that the chlorophyll level (34.53) at the initiation stage may be key in predicting tasseling success. Because the correlation coefficients for chlorophyll levels were also positively significant ($r = 0.80$) with the nitrogen treatment at initiation, the index level (critical level if using a chlorophyll meter) should be the chlorophyll level that occurred at the initiation stage (34.53). Any chlorophyll level at the initiation stage of sugarcane reproduction should not be any greater than 34.53 (SPAD reading),

Table 9. Mean leaf chlorophyll levels and correlation coefficients between leaf nitrogen and chlorophyll levels and chlorophyll and tasseling for two nitrogen treatments (nitrogen, 1; no-nitrogen, 2).

Variable	Treatment	Stage‡ 1	Stage 2	Stage 3	Stage 4
Chlorophyll Levels	1	37.80	34.53	38.45	37.43
	2	29.82	38.94	42.94	38.43
Correlation coefficients: chlorophyll vs. nitrogen	1	0.59*	0.80*	0.85*	0.53*
	2	0.69*	0.73*	0.51*	0.60*
Correlation coefficients: chlorophyll vs. tasseling†	1	0.05	-0.24	-0.16	-0.16
	2	-0.07	-0.23	-0.05	0.01

† Based on percent tasseling.

* Represents that the model is significant at $\alpha=0.05$.

‡ Stage 1, 2, 3, and 4 correspond to the vegetative, initiation, elongation, and anthesis respectively.

because by virtue of the relationship between chlorophyll and nitrogen this data indirectly suggests an inverse relationship between chlorophyll level and percent tasseling (Table 9). The SPAD reading (34.53) of chlorophyll coincides with the significant negative associations of leaf nitrogen and tasseling at the initiation stage.

SUMMARY

Nitrogen and no-nitrogen fertilizer treatments were used to study nitrogen effect on tasseling and other leaf macronutrient levels in various stages of sugarcane subjected to artificial photoperiod regimes. The plants in this study were grown in pots which provided one of the first attempt to look at nutrient contribution to tasseling in potted plants. Comparable studies with critical leaf nutrient levels were available only from field grown plants and therefore, only allowed for comparison with the vegetative stage in this study.

Nutrient levels were generally higher in the no-nitrogen compared to the nitrogen treatment. Stalk numbers were generally lower in the no-nitrogen compared to the nitrogen treatment which probably resulted in a higher nutrient uptake on a per plant basis for the no-nitrogen treatment. This is a caveat in the study because it may unduly influence interpretation of the influence of nitrogen on the other nutrients. Among the nutrients examined (N, P, K, S, Ca, Mg), the influence of nitrogen was significant ($P < 0.05$) only for P and S. Tasseling in the nitrogen and no-nitrogen treatments were 77% and 25%, respectively. The only meaningful association between nutrient and tasseling was a negative one found for nitrogen at the initiation stage. To the extent that 77% flowering was achieved in this study then, these leaf nutrient levels for the nitrogen treatment could serve as a guideline for the LSU AgCenter Sugarcane Breeding Program pending the results from future, more refined studies.

A chlorophyll meter was used as an indirect and cheaper way to measure leaf nitrogen. Strong correlations ($P < 0.01$) were found between chlorophyll and nitrogen levels for all stages. Although non-significant, the correlations between chlorophyll levels and tasseling were similar in trend to that between nitrogen and tasseling. Chlorophyll monitoring is a lot less expensive and less time consuming than the typical leaf sample analysis that has traditionally been used to monitor for nitrogen levels.

Suggestions for bettering this study could include individual leaf samples for each stalk of every genotype throughout the sugarcane life cycle. This leaf sampling and leaf analysis method would require considerably more amount of money for nutrient analysis but would give more conclusive results based on individual genotypes. Additional studies could compare the nutritional status of sugarcane genotypes grown in pot culture not undergoing an artificial photoperiod regime to sugarcane genotypes grown in pot culture undergoing an artificial

photoperiod regime. This type of study may be able to develop decisive critical nutrient levels for reproductive sugarcane grown in pot culture. For proper macronutrient analysis besides nitrogen, a better study would differentiate specific macronutrient treatments designed to study the effect of that macronutrient on percent tasseling. The main limitation for all studies regarding pot culture undergoing an artificial photoperiod regime is the amount of space available for treatments to be administered. This type of study will always hinge upon the purpose that the artificial photoperiod regime was meant for, commercial seed production.

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CHAPTER 4

TEMPERATURE EFFECTS ON SUGARCANE TASSEL PRODUCTION UNDER ARTIFICIAL PHOTOPERIOD REGIMES

INTRODUCTION

Cross hybridization remains the foremost means through which sugarcane breeders create genetic variation for selection. Sugarcane tassels naturally and profusely under tropical conditions. Unfortunately, sugarcane does not tassel naturally under the temperate conditions in Louisiana because winter temperatures are generally too low. The LSU AgCenter's Sugarcane Breeding Program must rely on artificial photoperiod regime to achieve tasseling. Desirable parents must tassel at the same time for cross hybridization to occur without resorting to pollen storage. However, tasseling can be erratic even after artificial photoperiod regime, thus, a better understanding of the effects of other factors besides photoperiod regime would be helpful in predicting the tasseling characteristic of parents and in synchronizing crosses.

Photoperiod and temperature are two principal factors controlling the transition from vegetative to reproductive growth in grasses and legumes (Heide 1994). Between the two factors, photoperiodism is better understood than temperature. When photoperiodism in plants was discovered by researchers at the U.S. Department of Agriculture in the 1920's, this laid the foundation for further research on photoperiodic responses in several crop species (Taiz and Zeiger 2002). By 1949, knowledge had accrued about the photoperiod conditions necessary for sugarcane to tassel which made it possible to hybridize sugarcane in temperate regions. Temperate regions relied on artificial photoperiod chambers to induce tasseling which opened many avenues for other related research (Chilton and Paliatseas 1956). The construction of photoperiod chambers, for example, made it possible to fine tune the knowledge about light control. However, knowledge about temperature requirements remained elusive as temperature

could be controlled in most photoperiod chambers only at night. During the day the plants are placed outside where they experience ambient temperatures.

Both night and day time temperatures are important factors in promoting the physiological change from vegetative to reproductive phase in sugarcane. Nuss (1980) reported the optimum night temperature for floral development to be around 23° C. At the LSU AgCenter's Sugar Research Station, night temperatures have been controlled through the use of heaters or exhaust fans and thermostats installed in each photoperiod bay, whereas, daytime temperatures have yet to be controlled.

Sugarcane is a short day plant requiring the dark period to exceed a certain critical length for a sustained number of days for tasseling to be induced. Any amount of light administered to the plants during this critical time period would interrupt and delay tasseling. In the LSU AgCenter's Sugarcane Breeding Program the dark critical time period is achieved by moving the plants into the photoperiod bay and controlling the temperature in the bay. Daytime temperatures, however, are more difficult to control. Research has shown that daytime temperatures above 32.2° C during the start of initiation inhibited tasseling (Moore and Nuss 1987). Although sugarcane is a C₄ plant and is relatively better suited to withstand higher daytime temperatures, the optimum daytime temperature differs depending on the stage of growth in the sugarcane life cycle. Vegetative growth temperatures are optimum at approximately 34° C (Irvine 1983); tassel initiation growth temperatures are optimum at 28° C (Miller and Li 1993); and anthesis or pollination stage temperatures are optimum at 26.6 - 29.4° C during the day (Paliatseas 1976).

Very little is known about the effect of ambient temperatures on tasseling in the LSU AgCenter's Sugarcane Breeding Program. Several years of historical data are now available

from the LSU AgCenter's Sugarcane Breeding Program to permit an analysis of the effect of ambient temperature on sugarcane tasseling. Such knowledge may be helpful in predicting the tasseling behavior of genotypes based on ambient temperatures which would facilitate efforts to synchronize tasseling.

The main objective of this research was to examine the relationship of daytime maximum temperature on the induction phase of reproduction and how it relates to sugarcane tasseling. Through regression analysis, prediction equations were developed for forecasting percent tasseling for the sugarcane breeding genotypes. The results of this research may lead to future solutions for restructuring the artificial photoperiod regime or the development of facilities to manipulate temperatures.

MATERIALS AND METHODS

Design of Experiment

Historical tasseling data collected over a 14 year period, from 1993 to 2006, at the LSU AgCenter's Sugar Research Station (latitude 30° 15' N, longitude 91° 05' W at an elevation of 5.79 m) in St. Gabriel, Louisiana were used in this study. Different regimes where photoperiod treatments are started at different times was adopted to avoid overwhelming the crossing program should a tasseling peak occur. Tasseling data from two different photoperiod regimes over a 12 year period from 1995 to 2006 were used in this study. Tasseling data in this study represent percent tasseling for each year. The tasseling data represent sugarcane breeding genotypes subjected to normal cultural practices in the LSU AgCenter's Sugarcane Breeding Program's "Photoperiod and Crossing" stage (LaBorde et al. 2004). Tasseling information for this experiment were from archived records in the LSU AgCenter's sugarcane breeding records

which have been published on a yearly basis (beginning in 1992) in the LSU AgCenter's Sugarcane Research Annual Progress Report.

By the end of the sugarcane breeding season all sugarcane breeding genotypes have been examined for signs of initiation. The sign of tasseling is when successive leaf sheaths become longer and blades become shorter. The terminal meristem, which is surrounded by a leaf sheath, ceases to form leaves and develops into an inflorescence primordial about three months before tasseling (Blackburn 1984).

Data Collection

Historical data collected from 1993-2006 included overall percent tasseling and average daily maximum temperature for specified time intervals for the months of May, June, July, August, and September. Data collected from 1995-2006 included overall percent tasseling and average daily maximum temperature for the same specified time intervals for both an early-tassel and a late-tassel artificial photoperiod regime. The dependent variable for this study was percent tasseling. Percent tasseling represents the percent of genotypes that tasseled in each year. Sugarcane breeding genotypes in the LSU AgCenter's Sugarcane Breeding Program changes each year because of recurrent selection for improved parents and the limited space available for the artificial photoperiod regimes. The specified time [May, June, July, August, and September] periods that were selected coincide with physiological developmental stages that are required for inducing a phase change from the vegetative to the reproductive growth stage under an artificial photoperiod regime. The daily maximum temperature data for the 14 year period under study were collected from a database called the Louisiana Agrilclimatic Information System (Anonymous. 2002). The temperature data from May 30 to September 10 for each year were extracted from the data set.

Statistical Analysis

The data were analyzed using multiple linear regressions. As described by Freund and Wilson (2003), the statistical model for analyzing a multiple linear regression is of the form:

$$y = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \dots + \beta_m\chi_m + \varepsilon$$

where, y is the dependent or response variable, and $\chi_i, i = 1, 2, \dots, m$ independent variables. The β_i are the (m) parameters or regression coefficients for each independent variable, β_0 is the intercept, and ε is the random error. In this analysis, the dependent variable (y) were the overall percent tasseling, percent tasseling early, or percent tasseling late which is representative of the three different artificial photoperiod regimes. The independent variables, $\chi_i, i = 1, 2, \dots, m$, represented daily maximum temperatures for specified time periods.

The temperature ranges chosen for the study consisted of the following: average daily maximum temperature from May 30 to June 14 (X_1), average daily maximum temperature from June 15 to June 30 (X_2), average daily maximum temperature from July 1 to July 15 (X_3), average daily maximum temperature from July 16 to July 31 (X_4), average daily maximum temperature from August 1 to August 15 (X_5), and average daily maximum temperature from August 16 to September 10 (X_6).

The data were subjected to regression analysis by using the PROC REG statement in SAS. The PROC REG procedure fits least-squares estimates to linear regression models. A complete model for all artificial photoperiod regimes was fitted in addition to individual models for particular artificial photoperiod regimes. All complete models were reduced by certain variable selection techniques (R-Square, Backward elimination, Forward selection, and Stepwise) until the reduced model was optimum (Freund and Wilson 2003). Significance levels ($P \leq 0.10$) were set at defaults for all models. Criteria for selecting the best reduced model

included the model with the smallest C_p (a statistic that measures random quantity with an error) and largest R^2 values. In addition to selecting the best model, a regression diagnostic was done for checking statistical assumptions, multicollinearity, and confidence intervals to validate the reduced model (Muller and Fetterman 2003). In analyzing the data, the fact that a regression relationship has been found to exist does not imply that x causes y if there are multiple factors affecting the incidence of sugarcane tasseling. Freund and Wilson (2003) have cautioned against using an estimated regression relationship for extrapolation purposes beyond the range of the x values.

RESULTS AND DISCUSSION

This study encompassed six independent (predictor) variables that were used to quantify the relationship between daily maximum ambient temperature and percent tasseling for the LSU AgCenter's Sugarcane Breeding Program (Table 10). The predictor variables, averaged daily maximum temperatures, were separated into various critical timeframes that began with vegetative growth (X_1) and ended with the termination of the artificial photoperiod regimes (X_6). The response variable, percent tasseling, was drawn from three artificial photoperiod regimes namely, early, late and overall which combined the early and late data sets regardless of when the photoperiod treatment commenced.

For the overall tasseling, the long-term average maximum temperature from 1993 to 2006 for X_1 was 32.2° C; for X_2 was 32.2° C; for X_3 was 32.8° C; for X_4 was 33.3° C; for X_5 was 33.3° C; and for X_6 was 33.3° C with an overall tasseling percentage of 53 percent (Table 10). For the percent tasseling pertaining to the early and late tasseling artificial photoperiod regimes, the long term maximum temperature from 1995 to 2006 for X_1 was 32.2° C; for X_2 was 32.2° C; for X_3 was 33.3° C; for X_4 was 33.3° C; for X_5 was 33.3° C; and for X_6 was 33.3° C with a

Table 10. Maximum temperatures and tasseling percentages of LSU sugarcane breeding genotypes.

Year	Average Maximum Temperatures (Celsius)						Percent Tasseling§		
	May 30 - June 14	June 15 - 30	July 1 -15	July 16 - 31	Aug. 1 -15	Aug.16- Sept.10	Overall	Early Tasseling†	Late Tasseling
	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆			
1993	32.2	31.1	32.2	33.3	33.9	33.3	76	.	.
1994	30.6	31.7	32.2	31.1	31.7	31.1	61	.	.
1995	31.7	31.7	32.8	33.3	32.8	33.9	30	20	28
1996	31.1	32.2	33.3	32.2	31.7	31.1	76	78	83
1997	30.0	31.7	33.9	33.3	32.8	32.8	45	48	52
1998	33.9	33.9	35.6	35.0	35.0	33.9	53	36	49
1999	32.2	31.7	32.2	33.3	35.0	34.4	68	67	65
2000	32.8	32.8	33.9	34.4	33.9	35.6	38	29	50
2001	30.0	32.2	32.8	33.3	32.2	31.7	55	58	44
2002	33.3	31.1	32.8	33.9	32.2	32.8	73	69	74
2003	32.2	31.7	32.2	33.3	33.3	33.3	48	33	63
2004	32.2	31.7	32.8	33.9	32.8	33.3	49	40	51
2005‡	31.7	33.9	33.9	33.9	33.9	34.4	27	26	28
2006	33.3	34.4	31.7	32.8	34.4	32.8	45	54	41
¥	32.2	32.2	32.8	33.3	33.3	33.3	53	.	.
£	32.2	32.2	33.3	33.3	33.3	33.3	.	47	52

† The percent tasseling data were not published into different artificial photoperiod regimes until the 1995 LSU Agcenter's Sugarcane Research Annual Progress Report.

‡ Data from one of the bays were omitted from the analysis due to mechanical failure from an extended period of time resulting in low tasseling percentage.

§ Overall represents all artificial photoperiod regimes, early and late tasseling represents those artificial photoperiod regimes designed to offset simultaneous tasseling in genotypes.

¥ Represents mean temperature for predictor variables from 1993-2006 for overall mean percent tasseling for years 1993-2006.

£ Represents mean temperature for predictor variables from 1995-2006 for early and late mean percent tasseling for years 1993-2006.

tasseling percentage equal to 47 percent and 52 percent, respectively.

The complete model for overall tasseling (Table 11) was significant (P=0.06) and accounted for 75% of variation in tasseling percentage (Table 12). The complete model was reduced by different variable selection techniques to determine the optimum subset or reduced

Table 11. Complete and reduced models depicting maximum temperature effects on the tasseling percentage of sugarcane breeding genotypes at Louisiana State University.

Artificial Photoperiod Regime	Model	Multiple Regression Models
Overall Tasseling	Complete	$Y = 438.92 + 3.49X_1 - 6.81X_2 + 2.19X_3 - 1.42X_4 + 5.48X_5 - 7.17X_6$
	Reduced	$Y = 501.42 + 4.19X_1 - 4.362X_2 - 4.69X_6$
Early Tasseling	Complete	$Y = 775.43 + 2.46X_1 - 4.55X_2 + 1.81X_3 - 5.35X_4 + 4.83X_5 - 7.11X_6$
	Reduced	$Y = 555.91 - 5.54X_6$
Late Tasseling	Complete	$Y = 654.50 + 5.26X_1 - 7.42X_2 + 5.39X_3 - 8.90X_4 + 2.65X_5 - 3.47X_6$
	Reduced	$Y = 506.25 + 3.82X_1 - 4.72X_2 - 4.02X_6$

model. When the reduced model was determined, it accounted for 60% of variation in tasseling percentage. The three significant variables for the reduced model included the daily maximum temperatures from May 30 to June 14 (X_1), June 15 to June 30 (X_2), and from August 16 to September 10 (X_6). These three variables had a significant effect on tasseling percentage for the overall artificial photoperiod regimes examined. The effect on tasseling due to these three variables was large enough to be measured above the random variation in the data (Schlotzhauer and Littell 2005). Based on the reduced model, percent tasseling was predicted to increase 4.19 percent if the daily maximum temperature goes up by one degree above 31.9° C during the May 30 – June 14 time period, decrease by 4.36 percent if the temperature goes up by one degree above 32.1° C during the June 15 – June 30 time period, and decreases by 4.69 percent if the temperature goes up by one degree above 33.1° C during the August 16 – September 10 time period. The $C(p)$ value of 7.00 was an indication of an adequate model based on Mallows (1973) suggestion that the value of $C(p)$ should not be too far or large above $p+1$ ($p=q-1$; q =number of predictors).

Although the initiation date varied between the early and late tasseling of the artificial photoperiod regimes, some conclusions can be made based on the overall tasseling percentage. Vigorous vegetative growth during the May 30 – June 14 time period was optimum at a temperature of 34° C (Irvine 1983). As temperatures increase into the low 32's (°C) for the May 30 – June 14 time period, floral evocation was enhanced. Early initiation coincides with the latter stages of June 15 – June 30 time period where temperatures in excess of 32.2° C have been shown to inhibit tasseling during the initiation phase (Moore and Nuss 1987). Temperature effects on floral development beyond the initiation stage of the time period August 15 – September 10 have not been reported. It was documented that vegetative reversions (a return to leaf production after a period of tassel development) in sugarcane tasseling was not possible past the 10th week of the reproductive developmental pathway (Moore and Nuss 1987). Alexander (1973) conveys a contradicting statement where vegetative reversion may terminate the tasseling process at any point up to actual emergence of tassels due to hormonal control of the tasseling and vegetative stimuli. Our data suggest that extreme post-initiation temperatures during the

Table 12. The analysis of variance, miscellaneous statistics, and C(p) values.

Artificial Photoperiod Regime	Model	P-value	R² Value	Adjusted R² Value	C(p) Value
Overall Tasseling	A†	.06*	0.75	0.54	7.00
	B‡	.02*	0.60	0.48	5.16
Early Tasseling	A	.38	0.62	0.16	7.00
	B	.02*	0.43	0.37	-.505
Late Tasseling	A	.27	0.68	0.30	7.00
	B	.10	0.52	0.34	3.57

† A represents the complete model for that particular artificial photoperiod regime.

‡ B represents the reduced model for that particular artificial photoperiod regime.

* Represents that the model is significant at $\alpha=0.10$.

August 15 – September 10 time frame may have a significant negative effect on sugarcane tasseling causing a vegetative reversal or the abortion of the inflorescence.

The early tasseling artificial photoperiod regime was analyzed independently to draw conclusions on reproductive sugarcane undergoing initiation at this time. Of all the years regarding the early photoperiod regime, the highest year for tasseling was 2002 (69%) whereas the lowest year was 1995 (20%). Because the complete model test was not significant ($P=0.38$), it fit the data no better than the reduced model using the dependent variable mean alone. It would be futile to seek a reduced model which adequately fits the data when the whole model is inadequate. Although a significant model could have been expected to support past research that quantified the negative effect of extreme high temperatures during initiation, a small sample size may have decreased the reliability of the regression model. At a minimum, a regression model requires a sample size of $N-q>0$ (N =number of samples; q =number of predictors). A further requirement for a stable estimate of error variance requires a sample size of $N>10 + q$. The number of samples for this model was smaller than the number of samples in the complete model regarding overall tasseling making this model more unreliable.

The late tasseling artificial photoperiod regime was also analyzed independently to draw conclusions on the reproductive sugarcane undergoing initiation at this time. Of all the years regarding the late photoperiod regime, the highest year for tasseling was 1996 (83%) whereas the lowest years were 1995 and 2005 (28%). Because the whole model test was not significant ($P=0.27$), no conclusion (Anonymous 1984-2003) could be drawn from this analysis. Again, the sample size may have been too small to draw meaningful inferences from the data set leaving the overall data set as the most reliable.

Of all the years, the overall tassel production was highest in 1993 and 1996 (76%) and lowest in 2005 (27%) and 1995 (30%). Based on the reduced model, the critical temperatures for X1 (May 30 – June 14), X2 (June 15 – June 30), and X6 (August 16 – September 10) were below 31.9° C, above 32.1° C, and above 33.1° C, respectively. The only year that conformed to all three critical temperatures for each time period simultaneously was 2002 resulting in excellent tasseling (73%) (Table 10). The only year that did not conform to the three critical temperatures for each time period simultaneously was 2005 resulting in poor tasseling (27%) (Table 10). The rest of the years conformed to various combinations of, but not all of, the three critical temperatures for various time periods.

Among the progenitor species of sugarcane, *S. spontaneum* genotypes tend to flower freely (without artificial photoperiod intervention) in several different environments including Louisiana, whereas *S. officinarum* seldom flowers. Because all commercial sugarcane breeding genotypes are hybrids of the original *Saccharum* species and in addition are not native to Louisiana, tasseling is very sporadic even following artificial photoperiod treatments. For some genotypes, tasseling never or seldom occurs even under artificial photoperiod regimes at the LSU AgCenter's Sugar Research Station. Thus, although variations are to be expected, an average overall tasseling percentage of fifty percent is considered to be good enough to achieve crosses in the LSU AgCenter's Sugarcane Breeding Program.

SUMMARY

Averaged daily maximum temperatures during certain time periods namely, May 30 – June 14 (X₁), June 15 – June 30 (X₂), and August 16-September 10 (X₆), was examined using regression analysis to determine their effect on percent tasseling. These time periods correspond to certain developmental stages during the transition from vegetative to reproductive growth of

sugarcane subjected to artificial photoperiod treatments in Louisiana. The response variable, percent tasseling, was drawn from three artificial photoperiod regimes namely, early, late and overall which combined the early and late data sets. Only the regression model using the overall tasseling proved to be reliable for interpretation because sample sizes were too small for tasseling percent drawn from the early and late data sets.

A complete model was fitted to the overall tasseling data set. The complete model was reduced by certain variable selection techniques (R-Square, Backward elimination, Forward selection, and Stepwise) until the reduced model was optimum (Freund and Wilson 2003). The complete model for the overall tasseling regime was significant ($P=0.06$). When a reduced model was fitted to the overall tasseling data, the model accounted for 60% of the variation in tasseling percentage. The $C(p)$ values for both the maximum model (7.00) and the reduced model (5.16) is an indication that this was an adequate model. The reduced model ($P=0.02$) specified three specific variables in the overall model, May 30 – June 14 (X_1), June 15 – June 30 (X_2), and August 16-September 10 (X_6), to be the most responsible for the tasseling response observed in the data. The three variables coincided with the vegetative transition May 30 – June 14 (X_1), late vegetative to early initiation June 15 – June 30 (X_2), and post-initiation August 16-September 10 (X_6) phases of vegetative to reproductive growth. The results indicate that the percent tasseling is expected to increase 4.19 percent when the May 30 – June 14 (X_1) variable goes up by one degree above 31.9°C , decrease by 4.36 percent when the June 15- June 30 (X_2) variable goes up by one degree above 32.1°C , and decrease by 4.69 percent when the August 16– September 10 (X_6) variable goes up by one degree above 33.1°C . The time period August 16– September 10 (X_6) is rarely mentioned in the literature with regards to suppression of sugarcane tasseling. This post-initiation timeframe from August 16 – September 10 (X_6) may be

a key unknown factor inhibiting the tasseling of some sugarcane genotypes in the LSU AgCenter's Sugarcane Breeding Program. For the one year (2002) that did conform to all of the critical temperatures for each time period, excellent tasseling (73%) was achieved. For the one year (2005) that did not conform to any of the critical temperatures for each time period, poor tasseling was achieved (27%). All of the other years in the study were combinations of critical temperatures for the various time periods (X1, X2, and X6). The study showed that daily maximum ambient temperatures affected the outcome of sugarcane tasseling in the LSU AgCenter's Sugarcane Breeding Program. The inability to control daytime highs (temperature) at certain time periods during the artificial photoperiod treatment can be a factor limiting genotypes from expressing their full tasseling potential.

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