COMPARATIVE STUDY USING OPEN-TOP GROWTH CHAMBERS AND AMBIENT PLOTS TO EVALUATE THE EFFECT OF CHAMBER MICROCLIMATE ON SUGARCANE (*SACCHARUM SPONTANEUM* CV. US-67-22-2)

By

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ABSTRACT

Cylindrical open-top chambers are experimental facilities that have been widely used to study the effect of CO_2 and toxic gases on plants. The difference in the chamber environment constitutes unintentional effects of experiments using open-top field chambers. The objective of this research was to study the effect of open-top chambers (CH) on the physiology of sugarcane variety US-67-22-2 in comparison to ambient air plots (AA). The chambers were 4.66 m diameter and 3.6 m high with 0.02 cm (8mil) polyvinyl chloride film covering an aluminum frame. The experiment was conducted at Lajas Agricultural Experimental Substation in Lajas, Puerto Rico. The environment of the open-top chambers was found to differ from the ambient air plots. Photosynthetically active radiation was reduced by 18 to 20% inside the chambers due to the chamber film and aluminum frame. Mean air temperatures inside the open-top chambers were on average 1.3 ºC higher than ambient plots. Short term differences up to 2.5 ºC were also observed on sunny days. CO_2 concentrations were virtually the same for both treatments. The environmental changes were shown to cause little differences in plant development. Significant differences where found in plant height ($P < 0.018$) and in the number of leaves on the main stem ($P < 0.04$) of the sugarcane. The plant height in the chambers was taller by 34% from the ambient plots. No significant differences were observed in the number of tillers ($P < 0.15$), total leaf area ($P < 0.06$), leaf photosynthesis ($P < 0.10$) and leaf chlorophyll content ($P < 0.09$). No significant differences in any of the fresh and dry weight of plant parts were found between the CH and AA plots, although average weights were higher in the AA plots. Although there is some modification of the plant environment, the chambers provide a suitable environment during the growing season to be use in CO_2 studies. Mathematical models were used to study the agronomic variables (height, leaf area, number of leaves, and number of tillers). The models with the best adjustments curve in relation to days after planting and degree day were the sigmoid and quadratic for the respective variables.

RESUMEN

Las cámaras cilíndricas de tipo abierto son estructuras experimentales que han sido ampliamente utilizadas para estudiar el efecto del CO_2 y de los gases tóxicos en las plantas. La diferencia en el ambiente de las cámaras constituyen efectos no-intencionales al utilizar las cámaras de tipo abierto en experimentos. El objetivo de esta investigación fue estudiar el efecto de las cámaras de crecimiento de tipo abierto en la fisiología de la variedad de caña energetica US-67-22-2 y compararlo con respecto a condiciones ambientales. Las cámaras tenían un diámetro de 4.66 m y 3.6 m de alto con una cubierta de cloruro de polivinilo de 0.02cm (8 mil) que cubría el marco de aluminio. El experimento se realizó en la Estación Experimental Agrícola de Lajas en Lajas, Puerto Rico. El ambiente dentro de las cámaras de tipo abierto fue algo diferente comparado a las condiciones ambientales de afuera. La radiación fotosintéticamente activa fue reducida entre un 18 a 20% dentro de las cámaras debido al efecto del plástico y del marco de aluminio. La temperatura dentro de las cámaras fue de 1.3 ºC mayor comparado a las condiciones ambientales. La temperatura llegó a alcanzar diferencias de 2.5 ºC más dentro de las cámaras en algunos días soleados. Las concentración de CO_2 eran virtualmente iguales para ambos tratamientos. Los cambios ambientales causaron poca diferencias en el desarrollo de la planta. Se encontró diferencias significativas en la altura de la planta ($P < 0.01$) y en el número de hojas ($P < 0.04$) del tallo principal para la caña de azúcar. La altura de la planta en las cámaras fue mayor por un 34%. No se encontró diferencias significativas en el número de tallos ($P < 0.15$), área foliar total ($P < 0.06$), fotosíntesis ($P < 0.10$) y en el contenido de la clorofila ($P < 0.09$). No se encontraron diferencias significativas entre el peso fresco y seco de las partes de la planta, aunque generalmente fueron mayores los pesos en las condiciones ambientales. Aunque haya una cierta modificación del ambiente de la planta, las cámaras proporcionan un ambiente conveniente durante la época de crecimiento para ser utilizados en estudios de CO_2. Se utilizaron modelos matemáticos para estudiar las variables agronómicas (altura, área de la hoja, número de hojas, y número de tallos). Los modelos que mejor ajustaron en lo referente a días después de siembra y días de calor acumulado fueron el sigmoide y cuadrático para las respectivas variables.

DEDICATION

To God for being my most sincere friend and giving me the strength to complete most of my dreams which this one was not the exception. To Him is the glory and honor, and power, and might for ever and ever.

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LIST OF ABBREVIATIONS AND SYMBOLS

Symbol	Definition
AA	Ambient air plots
CEER	Center for energy and Environment Research-University of Puerto Rico
CH	Chamber Treatment
CO_2	Carbon dioxide (µmol mol^{-1})
DAP	Days after planting
EEA-UPR	Estacion Experimental Agrícola de la Universidad de Puerto Rico
EPA	Environmental Protection Agency
FACE	Free- Air CO_2 Enrichment
DD	Growing Degree day
IPCC	Intergovernmental Panel on Climate Change
IRGA	Infrared gas analyzer
LAI	Leaf Area Index
LPM	Liter per minute
NF	Non-filtered
OTC	Open-top chamber
PAR	Photosynthetically Active Radiation (µmol $s^{-1}m^{-2}$)
ppm	parts per million
PVC	polyvinyl chloride film
Tbase	Base Temperature (ºC)
Tmax	Maximum temperature (ºC)
Tmin	Minimum temperature (ºC)
µmol mol^{-1}	Mole fraction of CO_2

1. INTRODUCTION

The earth climate has changed over the last century. The industrial and agricultural activities performed by man have had great influence on the climate as a result of changes in the earth surface and the composition of the atmosphere (IPCC, 2001). The phenomenon called "global climate change" is very important because of its potentially negative effects on the planet, in large part due to the constant increase of greenhouse gases in the earth atmosphere. Carbon dioxide (CO_2) alone represents a 60% of the global warming potential of well-mixed greenhouse gases (Hansen et al., 1998).

Since the beginning of the industrial revolution in the late eighteenth century, the atmospheric CO_2 concentration has increases from about 290 µmol mol^{-1} to the current level of more than 360 µmol mol^{-1}. The concentration continues to rise by about 1.5 µmol mol^{-1} per year, mainly caused by fossil fuel burning and changes in land use (Houghton et al., 1992). This increase may have widespread effects on plant growth, development and productivity (Taiz and Zeiger, 1991). Since CO_2 is the substrate for plants photosynthesis, an increase in its atmospheric concentration is usually accompanied by an increase of the photosynthetic rate, yield, growth and biomass of most plants (Stitt, 1991). The possibility to increase plant productivity and the interest of studying the response of plants to elevated CO_2 concentrations has generated a great deal of studies where the response of different plant species to elevated atmospheric CO_2 has been tested (Kimball, 1983; Rogers et al., 1983).

An open-top chamber (OTC) is an experimental device that has been widely used for many of enhanced CO_2 studies (Allen, 1990; Chowdury et al., 2005; Leadly and Drake, 1993). It presently constitutes the major source of information on field crop responses to rising CO_2 (Heagle et al., 1988). Originally open-top chambers were designed for exposing herbaceous crops to above-ambient concentrations of gaseous pollutants such as ozone (Heagle et al., 1973; Mandl et al., 1973). Normally the shape of the chamber is cylindrical with a rigid frame covered by transparent films. Blowers or

fans are installed at the lower side of the OTCs to provide a continuous high rate of ventilation to keep the inside temperature and humidity close to those of ambient air.

The first chambers developed employed a closed design (Thompson and Taylor, 1966; Hitchcock et al., 1963). Consequently, these chambers have not exposed the plants to the true ambient environment and have caused a number of problems. Temperatures reach 3 to 10°C higher than ambient; the transparent plastics used to cover the chambers alter the light spectrum and intensity of reaching the plants and it excluded rain, requiring artificial irrigation. Heagle et al., (1973) designed an open-top chamber to provide more natural conditions which would avoid or reduce most of the deficiencies of the close-top design, allowing direct sunlight and precipitation to enter through the open top. However it is believed that OTCs create artificial environmental conditions, and plants often grow differently inside than outside. It is known that in the OTCs temperature is typically higher than ambient by 1 to 3 °C (Heagle et al., 1988).

Open-top field chambers provide a relatively realistic ambient environment; however, they have slightly higher air temperatures, slightly lower light intensities, and a different air flow pattern over plant canopy compared to outside areas (Olszyk et al.,1980; Weinstock et al., 1982). On average, enlarged open-top chambers consistently altered microclimate conditions from ambient levels; air temperature is raised 1 to 3 ºC; relative humidity lowered by 3 to 5%; photosynthetically active radiation (PAR) was reduced by 10 to 20%; ambient rainfall was predominantly excluded; wind speeds throughout the day and night were maintained at low, constant rates, and soil moisture level was decreased (Olszyk et al., 1992). These alterations in microclimate are considered “chamber effects”. These effects in microclimate inside OTCs undoubtedly affect crop growth and yield. OTC grown plants are frequently different from those of the open field (Olszyk et al., 1986; Heck et al., 1984). In studies using free air CO_2 enrichment (FACE), conditions are less affected, but the wind pattern around the plants is still influenced which affects aerodynamic crop resistance and temperature difference

between crop and atmosphere, and operation cost are higher due to extra gas usage (Kimball et al., 1997).

This information suggests that open-top chamber studies can overestimate or underestimate losses to the extent that their environment does not reflect ambient conditions. It is important to understand at what point the environment modification produced by the chamber is acceptable for plant growth experimentation. The diversity of OTC designs and climatic conditions, in which chambers have been used, has stimulated research into the chambers microclimate to ensure valid comparisons of experimental data. More information is needed concerning the environment within open-top chambers if extrapolation between chamber results and true field occurrences are to be confidently made. Until now, much study on crop response to the environment has been done on temperate climates; little is known on field base environment control system in the tropics.

This study was designed to investigate the environment within open-top chambers used for CO_2 studies and determine the effects on plant growth on sugarcane variety US-67-22-2 in comparison to ambient conditions. The interactive effect of chamber and ambient conditions are studied to gain a more detailed understanding of the relationship between these two microclimates. This study will provide the baseline for future CO_2 studies and the effect of climate change in tropical agriculture.

2. OBJECTIVES

The general objective of this study is to quantify the effect that open-top chambers have on the physiology of plants grown inside such chambers.

The specific objectives of the study are:

1. To quantify changes in environmental parameters in open top-chamber and their effect on crops and compare it to crops grown in ambient conditions.

2. Quantify the physiological response of sugarcane variety US-67-22-2 grown in open-top chambers and explain it through mathematical models.

3. LITERATURE REVIEW

Scientists are becoming aware that environment plays such an important role in plant metabolism, growth and development. During the past 25 years there has been a large increase in the use of controlled environments in biological research (Downs and Hellmers, 1975). The use of field chambers have helped in these studies although it has raised numerous questions related to its advantages and problems encountered in growing plants on a modified microclimate.

Environment control for plant growth is not a particularly new idea. Centuries ago people began bringing exotic plants indoors to avoid cold weather damage. This practice led quite naturally to the development of the greenhouse. Off-season production of specialty crops in artificially warmed greenhouses developed over the years along with the use of such facilities in research programs. The large breakthrough came with the designing of the first phytotron in 1949 by F.W. Went (Went, 1957 cited by Downs and Helmers, 1975) at the California Institute of Technology. Since then, much progress has been made in improving the efficiency of such systems and the reliability of the equipment. Many researchers have introduced modifications in design and size, depending on the particular objective of their study.

Early chambers were used to study the effect of gaseous pollutants on plant response. These chambers were completely enclosed and were subject to significant alterations within the chamber environment. For example, Heagle et al., (1972) used an enclosed portable field chamber to study the effect of ozone (O_3) on two cultivars of midget hybrid sweet corn (*Zea mays L.* 'Golden Midget and 'White Midget'). The chamber was 2.44 m^2 and 1.94 m high at the back and 2.25 m high at the front. The temperature differential between the chamber air and the ambient air reached a maximum of about 5 °C for several hours on hot cloudless days, but was typically 1.5 to 3 °C during the day. A small portion of light spectrum was excluded by the chamber due to the Teflon film. Chamber humidity was equal to ambient humidity. Heagle mentioned the possibility

that plant sensitivity to ozone could be altered by environmental changes induced by field chambers. Still there is no evidence indicating that the chamber-caused environmental variations mentioned above would alter plant sensitivity to O_3.

3.1 Microclimate in open-top chambers

Open-top chambers (OTCs) have been adopted to provide an environment closely resembling ambient conditions. Numerous investigations have revealed that these alterations in chamber microclimate still persist with less extent than closed chambers. In a cooperative investigation with Environmental Protection Agency (EPA) and the North Carolina State University Heagle et al., (1973) design and evaluated an open-top field chamber using Tobacco plants (*Nicotiana tabacum* L., 'Bel W_3') as ozone indicators. The chamber was 2.4 m high and 3 m diameter. The temperature within chambers was similar to that in ambient air except on hot, cloudless days with little or no wind. On such occasions the temperature in chambers was slightly higher (2 °C) than ambient air. Measurements of relative humidity indicated no difference between the chambers and ambient air. Plants grown in un-enclosed plots were significantly shorter than plants in non-filtered or charcoal-filtered chamber plots. The number of leaves per plant was not affected. No significant differences in any fresh weight variables between un-enclosed and non-filtered plots were found, although average weights were always less in un-enclosed plots.

In that same year, Mandl et al., (1973) built and tested a light-weight open-top chamber to determine its efficiency for exclusion of ambient oxidants by growing susceptible plants in chambers with or without charcoal filters and in outside plots. The chamber consisted of three modules 1.22 m high and 2.74 m in diameter. Pinto bean (*Phaseolus vulgaris* L. cv. 'Pinto'), Tobacco (*Nicotiana tabacum* L.cv. 'Bel-W3') and a Perrenial rye-grass (*Lolium perenne L.*) where used as indicators. Temperature inside and outside of the chamber did not differ by more than 1 °C in the range of 16-29 °C. The greatest difference between the interior of the chamber and the external environment was

2 °C when the external temperature reached 35 °C. The greatest departure from natural environment was probably in the amount and distribution of solar radiation within the chamber. No significant differences between the rates of germination or dry weights of pinto beans where observed.

Heagle et al., (1979) found that temperature within chambers was usually similar to ambient air except for slight increases averaging 0.56 and 0.86 ºC on cloudy and sunny days, respectively. A maximum increase of 2.0 ºC was found in late morning on cloudless days. Cylindrical open-field chambers of 3 m in diameter and 2.4 m high were used at Raleigh, NC. Photosynthetically active radiation (PAR) in the chamber average 88% of ambient. The PAR was greater than ambient at some chamber positions at certain times during sunny days due to reflection from the plastic walls. Heagle used field corn (*Zea mays*) and spinach (*Spinacia oleracea*) to show the chamber effect on plant growth. The mean height of corn plants grown in ambient plots was 9% less than that in non-filtered chamber. Plant growth in one portion of a chamber could differ from growth in another portion. Their 7-yr experiences have shown that open-top chambers caused plants to grow slightly taller but rarely had significant effects on yield.

Olszyk et al., (1980) used an open-top field chamber (2.4 m high by 3 m in diameter) to asses the magnitude and significance environmental differences between open top field chambers and ambient conditions. Chambers were placed in a field planted with 'Vernal' alfalfa (*Medicago sativa L.*) at Madison, Wisconsin. Light intensity was 20% lower, temperature increases up to 2 °C were measured, and evaporative water loss was 10% less in chambers than open areas. Charcoal-filtered and non-filtered chambers have slightly different environments as a result of variations in air flow rates and dust accumulation. The environmental changes in chambers were shown to cause differences in plant growth which likely caused changes in pollution sensitivity of plants within the chambers. OTCs increase alfalfa yield.

A larger version of the 3 m diameter chamber was designed, constructed and tested by Heagle et al., (1989) as a tool to measure the effects of air quality on plant function and yield. This new chamber was 4.66 m diameter and 3.6 m tall and was equipped with a frustum (truncated cone) to decreased ambient air ingress. A frustum was included to improve exclusion efficiency. Nevertheless, some incursion is unavoidable, at a rate depending on wind speed (Heagle et al., 1973). During the daylight hours, the mean air temperature within the chamber was 0.6 °C greater than ambient on cloudy days, cold days, and 2.2 °C greater than ambient on partly cloudy, cool days, and 2.8 °C greater than ambient on sunny, warm days. The mean dew point temperature for a wide range of conditions was 0.7°C greater inside than outside. Mean solar radiation in the chamber, with new plastic panels was 15% less than ambient with a rain cap and 12% less with no rain cap. All measurements were performed in an empty chamber, except for grass which was kept mowed to a height of less than 10 cm. Heagle mentioned the need to measure chamber function and chamber effects on microclimate when plants of various types and sizes are growing in the chamber. Some evidence from the 3 m chambers indicates that plants sometimes grow different within chambers than outside. Alterations in climate and environmental factors of the magnitude caused by the chambers, may also alter the ways in which plants respond to pollutants, although no direct evidence support this. Experiments are needed to test this possibility.

Mandl et al., (1989) developed and tested two large open-top chambers (circular and rectangular) at Cornell University, NY to study the effect of air pollutants on large perennial plants. Chambers were tested in wind tunnel studies and subsequent in field trials. Flow visualization of air patterns in the wind tunnel showed that frustum and inner baffle plate covering 50% of the top surface provided the best exclusion capabilities. Light intensity was reduced 14 and 22% in the circular and rectangular chamber, respectively. In this series of experiments, the reduction of light did not seem to affect the vegetative growth or yield. Extensive monitoring showed that the large temperature differences were isolated instances and that mean differences were similar to those experienced in other design of open-top chambers (1 to 3 ºC temperature within chamber

compared to ambient). Mandle concluded that the conditions produced by the chambers were acceptable for plant growth and experimentation.

Weinstock et al., (1982) studied the performance of open-top chambers (3.0 m diameter by 2.7 m tall) on grapevines (*Vitis labruscana* Baily cv. Concord) at Fredonia, N.Y. The chamber environment was characterized by somewhat higher air temperature and decreased light intensity compared to ambient conditions. On sunny days, mean hourly chamber air temperature exceeded unenclosed temperature by 0.4 to 3.7 ºC. Air temperature generally decreased with increasing height on sunny days in control and chamber vines. The chamber environment was characterized by 5 to 10% lower relative humidity. Less light was received by chamber vines during early morning or late afternoon. Significant reductions in light intensity occurred in chambers due to reflection and absorption by walls and direct shading by aluminum framework. Growth of vines was not significantly affected by light depletion. Physiological studied showed little difference between chamber and non-chamber vines. Leaf temperatures were found to be higher inside the chambers. Stomatal resistance and water potentials readings on vines leaves showed no chamber effect on water status. Soil moisture availability was not affected by chamber enclosure.

3.2 Open-top chamber effects on plants

Researchers have assumed that the responses of plants growing within open-top chambers are similar to the response of plants growing under ambient environment. Although it has been pointed out that OTCs microclimate alterations can cause changes in growth and yield. Weigel et al., (1987) use open-top chambers with filtered and non-filtered air to study the effects of ambient concentrations of air pollutants on growth and yield of winter and spring barley. The experiment was located at Braunschweig, England. In the experiments with spring barley, fresh and dry weights of whole plants were lower and dry weights of leaves were higher in the filtered open-top chambers. Production of biomass of spring barley grown in ambient air was higher than that grown in OTC. Air

temperatures measured in the center of the chambers was on average about 1 °C higher than outside. However, short-term differences up to 2.5 °C were also observed on sunny days. Due to the shading effect of the chamber wall material, light intensity inside the chambers was generally 18-20% lower than outside. Weigel et al., (1987) concluded that although the chambers provided an environment closely resembling to outside field conditions, there was an effect of the chamber environment on the growth performance of both barley cultivars.

Sanders et al., (1991) reported larger leaf area of *Vicia faba* developed inside the OTC compare to plants grown in ambient plots. At harvest, chamber plants had a 13% higher yield than the plants grown in open field plots. Plant development progressed more quickly inside the chambers in response to the faster accumulation of thermal time. Chamber grown *Vicia faba* were up to 20 cm taller and had fewer branches than plants grown in ambient plots. Temperatures were on average 0.8 ºC higher inside the chambers compared with ambient air. Radiation was 20% less inside the chambers. The chambers use where of Raleigh, North Carolina design (3.1 m diameters, 2.4 m high).

In Northern Italy, Schenone et al., (1994) studied the effect of air pollution in open-top chambers (3.1 m diam, 2.4 m high) on bean (*Phaseolus vulgaris L.*). The study was made in two sites: urban and rural. Additional non-chambered plots were established to evaluate the chamber effect on plants. The comparison of plants grown in outside plots to those grown in non-filtered OTCs shows some differences in the measured physiological parameters. Photosynthesis of plants exposed to non filtered air was reduced by 40% in comparison to that of filtered plants. As a general trend net photosynthesis and transpiration were lower inside the chamber than outside. The differences in mean temperature among the treatments were not more than 1 ºC. Large differences in light intensity were detected between inside and outside the OTCs, due to the shadowing caused by the frame and the plastic sheets. These differences were greatest towards the end of the season (40% decrease inside).

Buckenham et al., (1982) used OTCs (hexagonal 2.4 m equivalent diameter, 2.3 m high) to determine the effect of air pollutants on spring barley. Un-enclosed plots were used to estimate the effect of open-top chambers on crop growth. The chambers were found to accelerate the crop's development by 7-8 days and reduce yield by suppressing tillering. The number of shoots per unit area was less inside the chambers by 36%. This may be due to the increased in temperature inside the chambers and a reduced in irradiance (10-20%). Buckenham reported an average increase in temperature of between 0.5 and 1.4 ºC at different time of day. Faster development and less dry matter growth in the chambers resulted in fewer ears and gain per m^2, and fewer grains per ear. However, weight per grain was about 16% greater in the chambers. Buckenham concluded that although the differences in temperature and light intensity between chambers and outside are small, they are able to affect significantly the growth of the crop.

Fuhrer (1994) studied the influence of OTCs on radiation, air temperature, and soil water content in relationship to plant growth and yield of managed pasture. Leaf area index (LAI) was slightly reduced in OTCs as compare to ambient plots. The total accumulated dry matter yield for all six growth periods was only 7% lower in OTCs. Average reduction in global radiation in OTC was 25%, and volumetric soil water content was reduced by about 5%. Daily mean temperature was increased by 1.3 ºC and the thermal time (degree days with base temperature of 5ºC) was increase by 12%. In OTCs the deficit in soil and atmospheric moisture was larger than in the open field, and the increase in daily mean temperature was strongly influence by the stage of canopy development.

At Beltsville, Maryland, from 1972 to 1976 and at Raleigh, North Carolina, Heggestad et al., (1980) studied the effect of snap beans (*Phaseolus vulgaris L.*) grown in filtered and non-filtered open-top chambers to assess oxidant (primarily ozone) induce yield reductions. Chamber environmental conditions alter in some way plant response. It was observed that plants were about 11-16% taller in chambers than in plots without

chambers. This could be due to temperature or light related factors. In North Carolina, the early Astro (fresh market bean) crop yielded significantly more in the ambient plots than in the filtered and non filtered chambers. Apparently, this was due to higher soil moisture in the open field and slightly elevated temperatures on sunny days in chambered plots. Light intensities were reduced due to the plastic coverings and aluminum frames. Photosynthetically active radiation (PAR) intensities in shaded areas were 50-60% of the brightest locations. Midsummer temperature in empty chambers or in those newly planted ranged from less than 4 ºC above ambient over relatively dry ground in high light locations to 2 ºC below ambient in moist shaded areas.

Olszyk et al., (1986) investigated the use of an air exclusion system for air pollution studies that provided less environmental modification compared to chambers. The experiment lasted two years. He saw the need to develop air pollutant exposure systems for evaluation of the true chamber effect with open-top field chambers. Alfalfa (*Medicago sativa L.*) was used as the test crop. Plant response was measured in air exclusion systems, open-top chambers (3.0 m diameter. and 2.4 m high), closed–top chambers, and outside plots. Results showed statistically differences in plant growth and yield with the different exposure systems. Plant growth was most similar between non-filtered air exclusion system chambers and outside plots, somewhat altered in open-top chambers, and greater in closed-top chambers than the other facilities. The air exclusion systems themselves had no effect on any alfalfa response parameter at any harvest based on comparisons between non-filtered systems and outside plots. In contrast, open-top chambers consistently affected alfalfa growth, as shown by the lower weights, greater heights, and greater injury in non-filtered open-top chamber compared to circular outside plots. During 1984, the closed-top ventilated chambers generally increased dry weight and height with respect to both other systems. The experiment indicated that with cooler weather, air exclusion systems produce plant growth more representative of the field environment than the conditions found in open-top field chambers.

Olszyk et al., (1992) studied the effects of open-top chambers on Valencia orange trees (*Citrus sinensis L*. Osbeck). The trees were exposed to ambient ozone (O_3) for 51 months in large (4.3 m diameter. by 2.9 m high) non-filtered open–top chambers (NF) and in ambient air without chambers (AA). The 19% PAR reduction in non-filtered chambers versus ambient plots was attributed to the position of the sensor near to the wall of the chamber. Specific leaf areas (cm^2 g^{-1}) were larger in NF than AA trees. In cool season net photosynthetic rates were 18% lower for NF than for AA trees. For both NF and AA trees, leaf gas exchange rates were higher in warm than cool seasons indicating greater physiological activity. Growth was significantly greater for NF than AA trees. Olszyk concluded that the altered microclimate likely was the key factor determining differences in response between NF and AA trees.

Fuhrer et al., (1989) developed a 3-year experiment studying the effect of ozone on spring wheat (*Triticum aestivum* L., cv. Albis) using open-top chambers (1.5m diam, 1.8 m height). Due to the changes in the environment that the chambers produce, Fuhrer also investigated the changes and related them to differences in growth, development and yield between plants grown inside and outside of open-top chambers. Temperature inside chambers was 2.1 ºC higher inside than outside. Solar radiation was reduced inside the chambers by 19%. In 1986, plants inside the chambers produced slightly higher straw and grain yield. The difference in grain yield was associated with larger grain weights. This increase could have been caused by higher temperatures in the chambers.

3.3 Open-top chambers used as CO_2 control tools

Atmospheric carbon dioxide levels are increasing and are predicted to double in the next century. Together with other greenhouse gases CO_2 traps heat and can potentially make the earth's surface temperature warmer by 3 to 4 ºC (Watson et al., 1990; Houghton et al., 2001). These factors could be determinant on crop growth and development and could have major impacts on the productivity of crop ecosystems (Baker et al., 1992; Kimball, 1983). It is generally conceded that plant species may have

different abilities to absorb CO_2 and thus have different compensation points of CO_2 utilization. Positive effects of elevated CO_2 on the growth and yield of a wide range of crops have been reported in the literature (Kimball, 1983).

It is not easy to alter CO_2 experimentally around a crop in the field. Most information about crop responses to elevated CO_2 is obtained from studies in greenhouses, laboratory controlled-environment chambers, and transparent field chambers, where released CO_2 may be retained and easily controlled. These settings provided the basis for projecting CO_2 fertilization effects on the major food crops such as maize, rice, sorghum, soybeans, and wheat.

In recent years, OTCs have been used to study the effect of elevated CO_2 concentrations in plants (Schüzt and Fangmeier, 2001; Ward et al., 1999). Temperature controlled studies could be done also with the use of OTCs. However, any controlled environment system for CO_2 and temperature research, with the possible exception of free-air enrichment (FACE) system, modifies the microenvironment around the crop with subsequent effect on bioprocesses and plant growth. The degree of this modified environment depends on the system used. A FACE system is able to provide near ambient conditions but poorer control. At present, two types of environmental control are thought to best maintain the link between a crop canopy and the natural microenvironment over a large sampling area: 1) the open-top chamber and 2) the FACE system.

Oijen et al., (1999) wanted to determine whether open-top chambers are appropriate tools to study CO_2 effects on plants, in spite of chamber-induced warming. They grew wheat (*Triticum aestivum L.*) in hexagonal open-top chambers (OTCs) with 1.5 m between parallel sides. Cooling techniques were used to create temperature levels (cooled and non-cooled). Radiation level in open-top chambers was 25% lower than ambient plots. The temperature in non-cooled OTCs was on average 2.8 °C higher than in ambient plots. In spite of using the cooling system chambers were slightly warmer than

ambient plots. Average ambient CO_2 levels were 365-380 µmol mol^{-1}. Plants grown and measured at elevated CO_2 showed an increase of photosynthesis by about 30%. There was no difference observed between ambient (cooled and warm) chambers and ambient plots. These measurements showed no effects of temperature at all. Grain yield was also increased by elevated CO_2. The effect was stronger in warm OTCs than in cooled OTCs. Most measurements still showed some difference between ambient plots and OTCs. Oijen et al., (1999) concluded that the use of OTCs in CO_2 experiments will lead to overestimation of CO_2 response relative to ambient conditions outside OTCs, because chamber warming will decrease LAI and possibly, increase leaf photosynthetic sensitivity to elevating CO_2. Average yield response to CO_2-doubling was twice as high in normal OTCs than in cooled OTCs.

In Montecillo Mexico, Sánchez-Espino et al., (2000) studied the effect of CO_2 enrichment on maize (*Zea mays L.*) and common bean (*Phaseolus vulgaris L.*) under field conditions. Two CO_2 concentrations were established, normal (360 µmol mol^{-1}) and the enriched one (600 ±50 µmol mol^{-1}). Two-open-top circular chambers, 3.0 m of diameter per 2.0 m height for common bean and 3.0 m in diameter per 3.0 m in height for maize were used. Treatments consisted of: Two controls with ambient CO_2 (360 µmol mol^{-1}), one growing outside and the other growing inside of the open-top chamber. The third treatment consisted in growing the two crops inside their respective chambers with a CO_2 concentration of 600 ±50 µmols mol^{-1}. Leaf area was affected only in common bean at high CO_2, although it was not significant. Leaf area in maize was not affected. Photosynthesis rate in maize under high CO_2 concentration was 48 and 38% higher with respect to the controls (C-inside and C-outside). At 80 days after sowing, the biomass production in maize plants grown in high CO_2 was 24% and 18% higher than in C-inside and C-outside, respectively.

Coleman and Bazzaz (1992) studied the combined effect of increased CO_2 and temperature on photosynthesis, growth and biomass allocation using C_3 (*Abutilon theophrasti Medic.*) and C_4 (*Amaranthus retroflexus L.*) plants. CO_2 levels were 400 and

700 µmol mol^{-1} and light and dark temperatures of 28°/22°or 38°/31°C. These treatments were randomly assigned to environmental growth chambers. Final biomass of Amaranthus was enhanced by elevated CO_2 at 28 °C but depress at 38 °C. Plants of both species grown under elevated temperature had substantially decreased reproductive allocation, increased allocation to stem biomass, and increased plant water flux at both CO_2 treatments. For Amaranthus at 38°C peak leaf area production was not affected by CO_2 treatment, but the rate of net leaf area loss accelerated under elevated CO_2 conditions and was accompanied by substantial reduction in plant–nitrogen content and leaf photosynthesis. This may have led to the reduced biomass accumulation of high CO_2 grown plants.

At the International Rice Research Institute (IRRI) in Laguna, Philippines, Moya et al., (1997) designed and operated a new environment control system using OTCs to study the impact of CO_2 and temperature on a rice ecosystem. Although field test demonstrated that the system could set and control temperature and CO_2 to ±10% precision for >90% of the time, changes in chamber microenvironment conditions did occur. The radiation environment inside an OTC chamber could differ by approximately 9% from that in the open field. Air temperature inside the ambient chamber was higher than that in the open-field by about 1 ºC in the daytime and about 2 ºC during the night time. Plants in the open field produced 12.5% more above ground biomass than plants in the ambient chamber.

At the Silsoe Research Institute Norris et al., (1996) studied a new advanced controlled ventilation open top chamber (CVOTC) which offers considerable advantages over the current continuously-ventilated open-top chamber. A recirculation of the air helps reduced the use of carbon dioxide. Full ventilation at a maximum rate of six air changes per minute limited the chamber temperature to 2 °C above ambient during periods of high radiation. This enables the chamber temperature to be maintained at an average of 1.6 °C above ambient. The mean light transmission was approximately 90%.

The air distribution provided uniform air speeds above 1 m s^{-1} which produces leaf boundary layer resistances comparable with those of plants in the open.

It is now known that chamber alter the response of plants to elevated CO_2 (Ainsworth et al., 2002). Despite being partially open to the atmosphere, important environmental differences remain. The transmission of sunlight is lower due to the transparent-walls of the chamber, temperature inside are warmer, water vapor deficit tends to be higher and alterations of air flow and rainfall occurs. As a result, the effect of the chamber on plants is often greater than that of elevated CO_2 (Mcleod and Long, 1999). To overcome these limitations, free-air concentration enrichment (FACE) was developed.

A typical FACE apparatus consists of a 20 m diameter plot within the crop field, in which CO_2 is released just above the crop surface on the upwind side of the plot. Wind direction, wind velocity and CO_2 are measured at the center of the plot. Computer controls then adjusts the positions and amount of CO_2 released at different points around the plot. FACE allows the exposure of plants to elevated CO_2 under natural and fully open-air conditions. The CO_2 fertilization used in models to project future yields were derived from enclosure studies conducted approximately 20 years ago. Long et al., (2006) observed that the stimulation of yield observed in FACE experiments fell well below (about half) the value predicted from chambers. No significant yield or biomass increases has been observed for C4 crops or C4 wild grasses at elevated CO_2 in FACE studies (Ainsworth and Long, 2005). This is in contrast to the large stimulation of yield for well-watered plants in chambers used to parameterize models. FACE experiment suggest that there will be a much smaller CO_2 fertilization effect on yield than currently assumed with the used of controlled chambers (Long et al., 2006).

Ainsworth and Long, (2005) made a review of results of large scale FACE experiments and compare and contrast the results of chamber based studies with those of FACE experiments. For example, in open-top chambers grain yield of wheat increased

27% on elevated CO_2 from 359 to 534 µmol mol^{-1}, but only a further 3% increase was observed when comparing plants grown at 534 to 649 µmol mol^{-1} (Fangmeier et al., 1996). A similarly smaller than predicted response has recently been reported for soybean grown at elevated CO_2 within the soy FACE experiment (Morgan, 2004). This discrepancy has wide importance as the chamber values have formed the basis for projecting global and regional food supply, and the stimulation attributed to elevated CO_2 has commonly been presumed to offset yield losses that would otherwise result from increase stresses, including higher temperature, elevated ground-level ozone and changes in soil moisture. If chamber experiments have overestimated the direct effect of increased CO_2, this would have a major impact on projections of future crop yields and wider implications for extrapolations from chamber studies to terrestrial ecosystem.

3.4 Sugarcane

Sugarcane is the main source of sugar in tropical and subtropical countries of the world. In the United States, agriculture represents almost 50% of the total economy, being sugarcane one of the main tropical crops (Salassi and Legendre, 2002). In Puerto Rico the sugarcane crop became the most important crop in the first half of the twentieth century (Scarano, 1993). By 1910, 118,000 acres of land were cultivated with sugarcane. After the commonwealth political status was established in the fifties, a new economic approach was followed transforming the agriculture economy to an industrial one. It was the sugarcane industry who mostly plunged deeply in the crisis, resulting in a constant declination. The higher costs of production, the low technology efficiency of the companies and the low yield production are among the reasons that contributed to the crisis of the sugar industry in Puerto Rico.

According to the census of agriculture of 2002 of Puerto Rico only 2,004 acre were harvested for that year. For that same year sugarcane represented only a 0.2% of the market values of crops sold. The government has agreed to privatize the industry and transfer Sugar corporation assets to sugar farmers and the two existing mills. Efforts are

being made to bring back the sugarcane industry which was fundamental for the development of the Island. Currently, various projects are been evaluated by the Department of Agriculture of Puerto Rico for the production of ethanol from sugarcane.

Sugarcane is the world's most living collector of solar energy, storing this energy in a huge quantity of biomass in the form of fiber and fermentable sugars. Since sugarcane production has become a second nature in many tropical developing countries it is advisable to explore the numerous alternative uses of sugarcane and its products and by-products. Scientists worldwide are trying to use sugarcane biomass as an alternative fuel either by direct combustion, fermentation of solids to ethanol or by fermentation of cellulose and hemi cellulose in the cane biomass. Investigations were conducted in the United States and Puerto Rico to study the viability of using sugarcane biomass for a bio-energy industry (Lorber et al., 1984; Mishoe et al; 1979).

A five year study was initiated in 1977 to study the production of sugarcane and related tropical grasses as energy crops (Alexander et al., 1982). This study was a contribution to the biomass energy program of the University of Puerto Rico Center for Energy and Environment Research (CEER-UPR). Final cost and energy balances analysis indicated that tropical grasses are unquestionably economic and reliable energy resource with multiple benefits when managed specifically as energy crops in a tropical environment. A major component of the energy concept was the development of alternative tropical grass species as supplemental biomass sources. During this period breeding and selection of new *Saccharum* progeny having superior biomass productivity was developed in a very modest level. It was confined to desirable parent clones with suitable flowering characteristics and superior dry biomass producers. A series of *S .spontaneum* and *S. sinense* clones that had already shown favorable biomass attributes were used. The most significant result of this trial was the emergence of the *S. spontaneum* hybrid US-67-22-2 as a superior candidate for biomass production.

The US-67-22-2 variety was known as “energy cane” for its tremendous potential for biomass production and has been studied in Puerto Rico as a biomass source for energy generation (Allison and Rios, 1988). It has excellent germination, rapid early growth with strong tillering and ratooning capability, and erect growth habit. It has a relatively low fiber content and average sucrose content. Plant crop data have revealed that variety US-67-22-2 produced the highest green matter yield at 130 tons/acre, with total dry matter at 41.9 tons/acre (Alexander et al., 1982). The highest yields for this variety are associated with an increased number and sizing of stems harvested per acre (Alexander, 1985).

Energy cane received nitrogen supplies that are higher than those of commercial sugarcane. The highest single yield for 12 months was 130.3 t/ha, recorded for variety US-67-22-2 receiving 448 kg elemental N/ha (Alexander et al., 1982). Although the highest dry matter yield was recorded for US-67-22-2 receiving 674kg N/ha year. Torres Justiniano (2005) studied the physiological response of US-67-22-2 to different nitrogen levels (0, 448, 896, 1344 kg N/ha). Results showed that 448 kg N/ha were sufficient to obtain an adequate plant cane yield at reasonable cost. The association of plant height, number of tillers, number of leaves, and leaf area with yield was consistent. Guindín-García (2003) evaluated four nitrogen levels (0, 51, 101, 202 kg/ha) to calibrate the crop simulation model CANEGRO for cane variety US-67-22-2. The aerial biomass accumulation did not showed significant differences during eight of the nine samples taken during the experiment. No significant difference was observed between treatments on the number of leaves. The model over predicted the accumulation of biomass possibly due to its limited ability to model water stress.

Torres Justiniano (2005) quantified the physiological response of sugarcane US-67-22-2 and developed mathematical relationships between nitrogen levels (0, 448, 896, 1344 kg N/ha) and climatological parameters. A monomolecular, negative exponential model was used. It was observed that the application of 448 kg N/ha is enough to obtained adequate plant cane yield, photosynthetic rate and relative chlorophyll content.

Plant height range from 71 cm at 90 DAP to 217 cm at 300 DAP at maximum level tested. The number of tillers ranged from 13 at 90 DAP to 53 at 300 DAP when no N was applied. The main stem leaf area fallowed a linear trend at every nitrogen level. The application of 448 kg N/ha produce the highest increase. Photosynthetic rate and chlorophyll content increase with N applications, although the growth stage apparently had no significant effect.

3.5 Temperature and Degree Days

Temperature is the most frequently measured variable of all the environmental factors when studying plants in the field or in growth chambers. Temperature affects directly through the control of biochemical reaction rates in the various metabolic processes and indirectly through the development of water stress caused by the physical process of transpiration. The growth rate, in common with the enzymatic reactions that control it, exhibits an increase that is almost linear for most plants as the temperature is increase (Down and Hellmers, 1975). The response varies with species on time-temperature basis. The different biochemical reactions that occur in a plant have different maximum, minimum and optimum temperature. The effects on development are most pronounced when temperature is near the minimum or maximum for plant growth.

According to Alexander (1973), temperatures from 28 ºC to 31 ºC (82 to 88 ºF) are satisfactory for the optimum growth and development of US 67-22-2. The day temperature might not exceed 32 ºC (90 ºF) while the night temperature might not be lower than 18 ºC (65 ºF). In Puerto Rico, Lugo-López and Capó (1954) found that cool night temperatures with relatively broad ranges between maximum and minimum values increased sucrose yield more than other climatic factors. Samuels (1965) describes cool temperature as a major factor in sugarcane ripening.

Temperature controls the developmental rate of many organisms. Plants require a certain amount of heat to develop from one point in their life cycles to another. The degree day's concept is used to estimate the growth and development of plants during the growing season. The basic concept is that development will only occur if the temperature exceeds the base temperature (T_{base}). The base temperatures are determined experimentally and are different from each organism. The base temperature is defined as the minimum temperature required for a plant to start its development. If the temperature is below the base temperature it doesn't accumulate degree days. To calculate degree days you must first find the daily mean air temperature. This is done by averaging the daily maximum and minimum temperatures. This can be calculated by equation 1.

$$T_{mean} = \frac{T_{\max} + T_{\min}}{2} \qquad (1)$$

Then, the base temperature is subtracted from the daily mean temperature calculated using the equation 2. In this equation DD represent the degree day's accumulation in Celsius, n the number of days and Tbase (10 ºC).

$$DD = \sum_{i=1}^{n} (T_{mean} - T_{base}) \qquad (2)$$

In this project same base temperature as Guindín -García (2003) was used. Guindín-García, (2003) used 10ºC as base temperature to calculate the degree days for sugarcane variety US-67-22-2. This base temperature was calculated by Inman Bamber (2001) to be use in the CANEGRO model.

3.6 Predicting models

At each developmental stage of a crop, agronomic and crop-specific variables vary; consequently, they determine the crop's behavior, help in providing an understanding of the influence of climate parameters on yield, interfere with agricultural planning, and determine the magnitude of physiological stress and final productivity. Statistical models have been used to analyze the crop growth base on different variables such as leaf area, tiller density and plant height. Sigmoid models have been used to describe the patterns of growth for most plants. It follows a relative slow growth rate with a growth increase as the plants become larger, reaching a maximum and then decrease as the plant matures. Alexander et al. (1985) used a sigmoid shape curve to explain the growth stages of energy cane. At the early stages the aboveground is relatively flat, rises steeply as the growth increases and starts to decline after maturation (Figure 1).

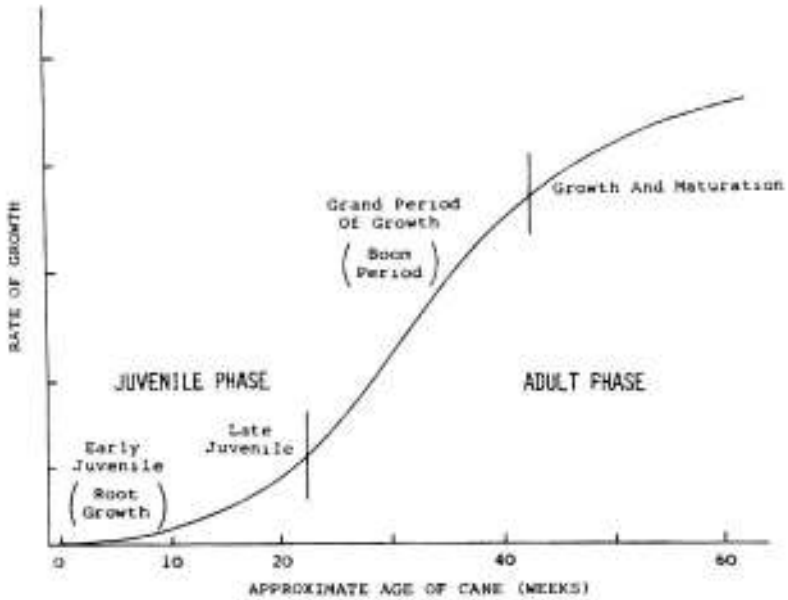


Figure 1. Juvenile and adult growth phase of energy cane plotted in a characteristic sigmoid curve. Adapted from Alexander, 1985.

Temperature is an environmental variable that have a major effect on the rate and duration of many developmental events in plants. The most widely use model of temperature, which predict phenological development, has been based on accumulated degree days (Slafer and Safin, 1991). Time can be express in thermal time unit, measured in units of degree-days (ºC-day) which is better plant time than day of the year and number of day after planting. It is evident that plant cycles are extremely affected by climatic variability, such as air and soil temperature, rainfall, solar radiation, and relative humidity that can provoke inconsistencies on the results base on days after planting or days after emergence. Accumulated degree day's is easier than a discrete variable, such as crop growth stages, for using on regression models because it provides a continuous and precise scale for independent variables, and because it can be use for comparing data, from different locations, years and sowing dates (Knezevic, S.Z. et al.,2003). In some cases the time of day has similar R^2 values as thermal time unit (Ferri et al., 2006).

Simoes et al., 2005 use linear and multiple regressions analyses to study the growth analysis and to correlate agronomic variables with biomass and productivity on sugarcane variety SP8-80 1842. A Gompertz model, a sigmoidal curve, was the best adjustment curve for total biomass and yield in relation to days after cutting (R^2=0.8987 and R^2=0.9682, respectively); number of plants and leaf area index showed best fit with a cubic exponential model and a quadratic exponential model, respectively. Total biomass and cane productivity were well correlated with leaf area index in the first two stages of sugarcane cycle using linear regression.

Torres Justiniano, 2005 used a monomolecular model to adjust the physiological variables and yield on sugarcane variety US-67-22-2 to different nitrogen levels (0, 448, 896, 1344 kg N/ha. The fitted model had the following equation $Y=\alpha\ (1-\beta e^{-\gamma x})$. To study the sugarcane yield for the different harvest intervals (240, 270 and 300 DAP) the regression equation used was:

Green Matter Yield =alfa*exp (-beta*exp (-gamma*treatment) +delta

For each harvest interval of 240, 270 and 300 days after planting the R^2 were 0.86, 0.79, and 0.98, respectively. This showed that the model fitted the observed data well.

To study the physiological variables such as height, leaf area, number of tillers, and number of leaves the best regression equation was:

Physiological variable=alfa*(1-beta*exp (-gamma*X)

This model was used to explain the different physiological variables in terms of days after planting and degree days. The best fit was obtained for the plant height which had values of R^2 =0.99 for each treatment for days after planting and degree days.

Ferri et al., 2006 characterize the structure of a *Panicum coloratum L. cv*. Verde pasture resulting from different forage accumulation periods, to assess if the rates of blade accumulation are constant on time and to determine the variable (chronological or thermal time) best correlated to predict this process. The results showed that the process that determine the rate appearance of leaves could be predicted using both the chronological time and the accumulation of degree days (base=10 ºC). Various authors have found that the total number of leaves as the rate of leaf appearance is associated more strongly with the accumulation of thermal time than with the chronological time (Wilhelm and Mcmaster, 1995). Although, in the present study the total number of leaves had R^2 values similar for both regressions. The regression equations for the relationship between leaf blades per tiller with growing days (t) and degree day (base 10 ºC) (GD) where as follow:

$3.9+ 0.0526t$	(R2=0.84)
$0.7+0.1886t - 0.012t^2$	(R2=0.97)
$3.8+0.0053GD$	(R2=0.87)
$0.8+0.0170GD-0.00001GD^2$	(R2=0.97)

4. MATERIALS AND METHODS

4.1 Experimental Site

A field experiment was carried out at the Lajas Agricultural Experimental Substation of the University of Puerto Rico (EEA-UPR) (Figure 2). The soil is a Fraternidad clay soil with a cation exchange capacity of 18 meq/100g clay soil. This soil is high in natural fertility and is neutral to alkaline. It is slowly permeable and difficult for tillage. The swelling and shrinking properties of this soil sometimes destroys young seedling. These soils have a very dark grayish-brown clay surface layer and brown clay underlying materials. The average annual rainfall is 1162 mm (45.75 in) (period of 57 years), with the wettest period from August to October and the driest season from January to March. The mean average maximum and minimum temperatures are 31 °C (88 °F) and 19 °C (66 °F), respectively, being a semiarid climate (SERCC, 1989). The annual evapotranspiration is 1803 mm (71 in/year) (Guindín García, 2003).

Life zones are climatic divisions that defines conditions for ecosystem functioning. These life zones are classified by using a triangular matrix whose axes represent mean biotemperature, precipitation and potential evapotraspiration ratio. Mean biotemperature is defined as the mean temperature modified by substituting zero for values outside the range of 0 to 30 ºC (Ewel and Whitmore, 1973). The life zones in Lajas are classified as a subtropical dry forest (df-S) and subtropical moist forest (mf-S) according to the Holdrige and Ewel and Whitmore (1973) life zone classification system. Much of the Subtropical Dry Forest zone in Puerto Rico lies in the warm and wet transitional parts of the life zones thus has characteristics of the dry part of the Subtropical Moist Forest and the cool portion of the Tropical Dry Forest. Example of such area is the Lajas Valley.

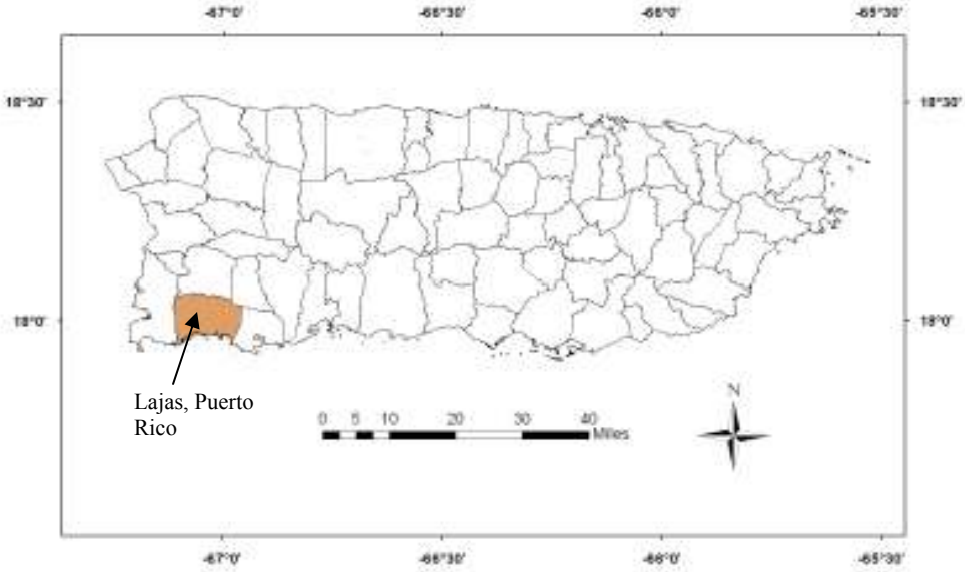


Figure 2. Map of Puerto Rico showing Lajas municipality.

4.2 Open–Top Chambers

The chamber used in this research project was of the Heagle design (Heagle et al., 1989), a cylinder type with an aluminum-channel frame covered with clear, 0.02 cm (8-mil) polyvinyl chloride film (PVC) containing UV inhibitors (Livingstone Coating Corp., Charlotte, NC) (Figure 3). The frame consist of four hoops, each 4.66 m in diameter (7.62 by 3.81 cm, structural channel), four 3.50 m vertical members (5.08 by 3.18 cm, structural channel), and eight diagonal (3.96 m) and five short (1.22m) vertical members (2.54 by 1.27 cm, architectural channel). The two upper cylindrical sections (upper two-thirds) of the chamber frame are covered with a separate, single layer of the clear plastic film. The lowest cylindrical section frame is covered by a double layer of film with six rows of holes (3.18 cm diameter.) on the inner layer (duct panel). The plastic panels are attached to the aluminum channel hoops (flanges face outward) with rope strung through a 5 cm wide channel at the margins of each panel. Previous work with 3 m diameter chambers showed that without a truncated cone (frustum), the efficiency of excluding ingress of ambient air through the open-top average about 50%. A frustum was used to improve exclusion efficiency. The frustum consists of a hoop 3.11 m

in diameter (5.1 by 2.5 cm channel) raised 79 cm above the chamber by 12 galvanized metal legs attached to the top main-frame hoop and bent inward at a 45° angle to decrease the size of the air outlet. The frustum frame is covered by a single layer of clear plastic film, leaving an opening of 3.11 m in diameter.

The duct panel is perforated on the inner wall with 468, 3.2 cm diameter holes 6 rows and 78 columns spaces 17.8 cm laterally and 15.2 cm vertically. The duct panel completely surrounds the chamber perimeter, except for a 114-cm wide section opposite the fan box that serves as the doorway. The doorway is covered by a single layer of the clear plastic film that serves as the door. The duct panel is connected to the fan box by a 1.5 m long, 94-cm diameter, and clear plastic-film duct. Each chamber was secured by four steel cable attached to the fourth highest 4.66 m hoop and metal screw-type anchors in the ground outside the chamber to avoid failure during strong winds.

A 3/4 (horse-power) propeller fan (Dayton, Model 3CC57 Grainger Industries, Inc.) connected to the duct panel moves air through the system at approximately 3800 L/sec (8000 cfm) providing about four air changes through the chamber per minute. The fan is housed in a galvanized sheet-metal box (102 cm wide, 102 cm tall and 69 cm long (Appendix A-Figure A1). Air passes through the connecting duct, inflating the duct panel, then through the 3.2 cm holes into the chamber and exits through the opening of the top frustum.

Three open-top chambers (OTCs) and three ambient air plots (AA) were randomly installed in a 557 m^2 area (30.5 m x 18.3 m) at Lajas Experimental Substation (Appendix A-Figure A2). Ambient plots where marked by aluminum frames (one chamber hoop) established on the ground having the same surface area as the chamber plots (Figure 4) (Appendix A-Figure A3). Studies made by the company with a spectrophotometer showed that the plastic covering is about 90% transparent in the visible region (Figure 5).

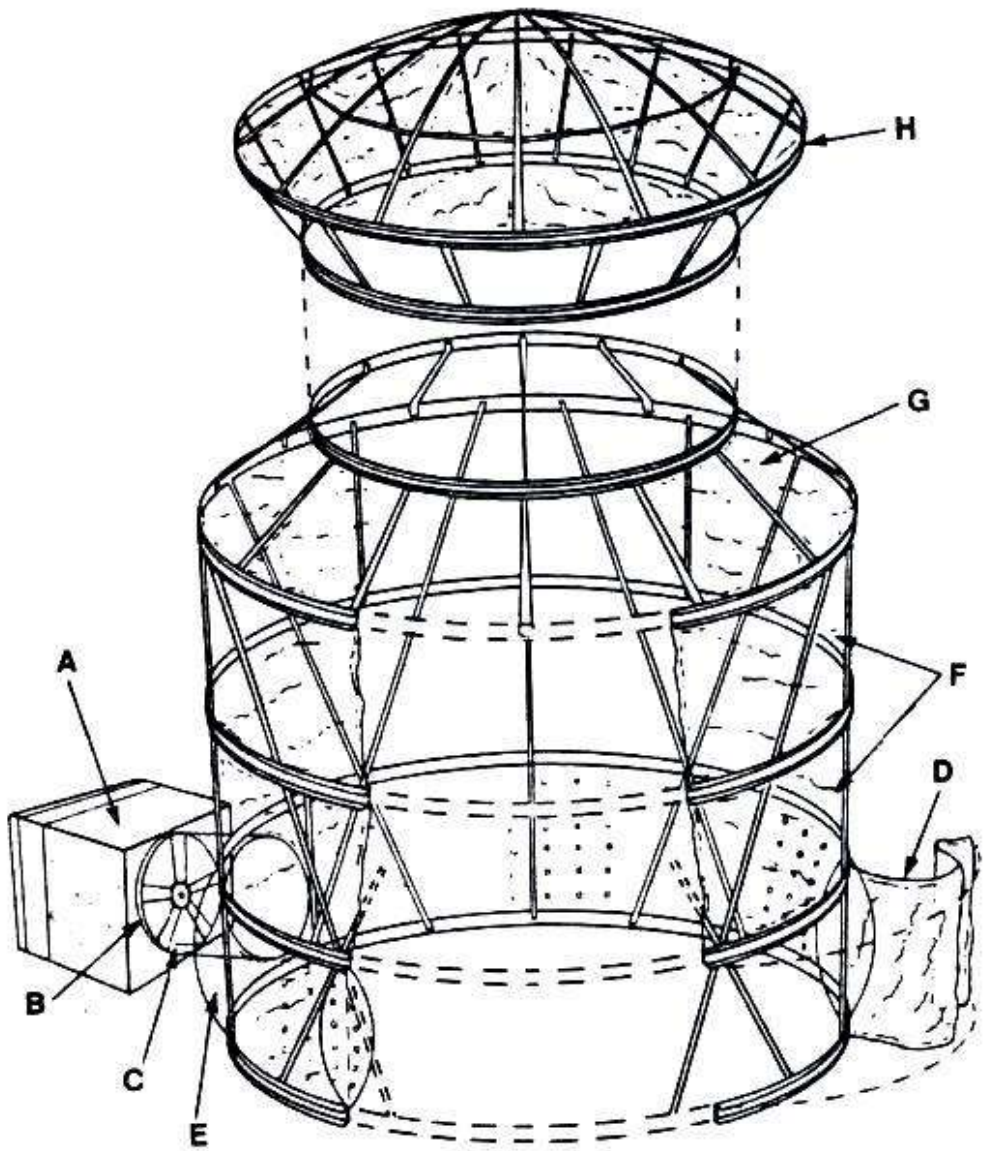


Figure 3. Open–top chamber schematic diagram. (A) galvanized-metal fan box; (B) propeller fan; (C) clear plastic connecting duct; (D) single-layer plastic door; (E) double–layer plastic duct panel (F) single-layer plastic panel (G) frustum (single layer plastic panel; (H) rain cap (not use). Adapted from Heagle et al., 1989.

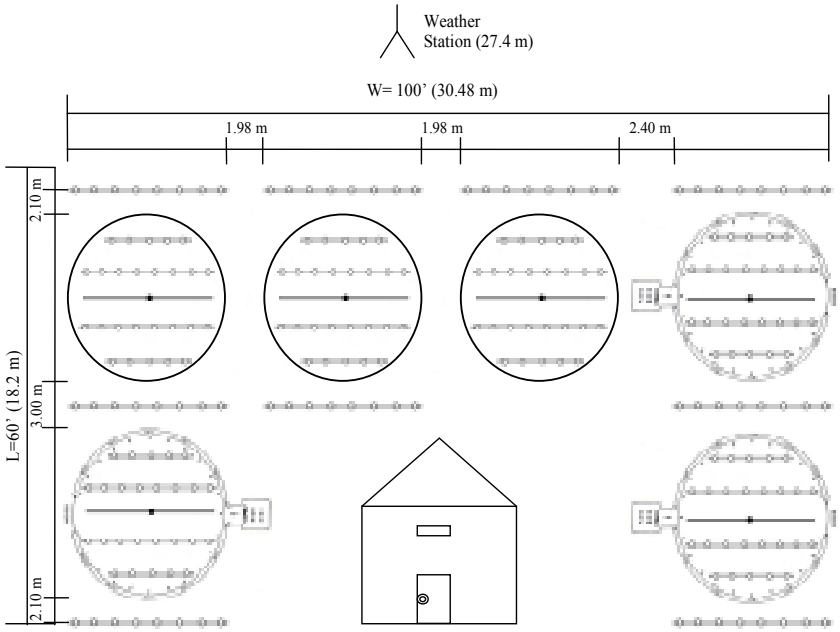


Figure 4. Diagram of the experiment setup

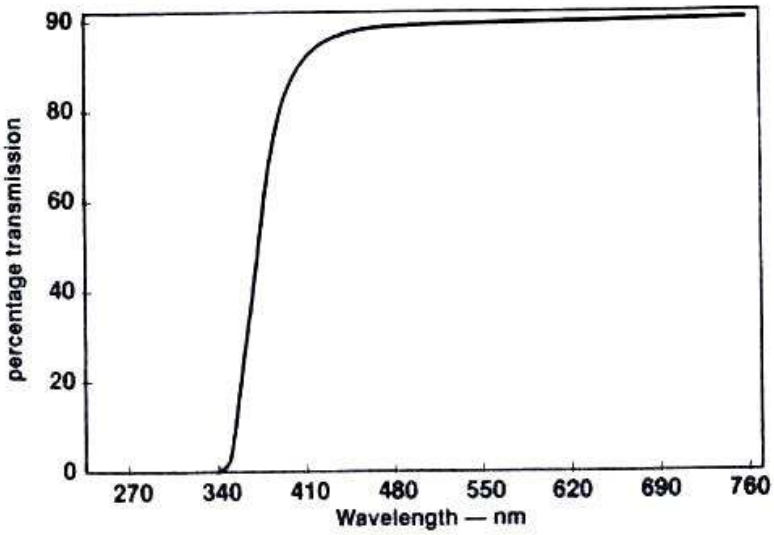
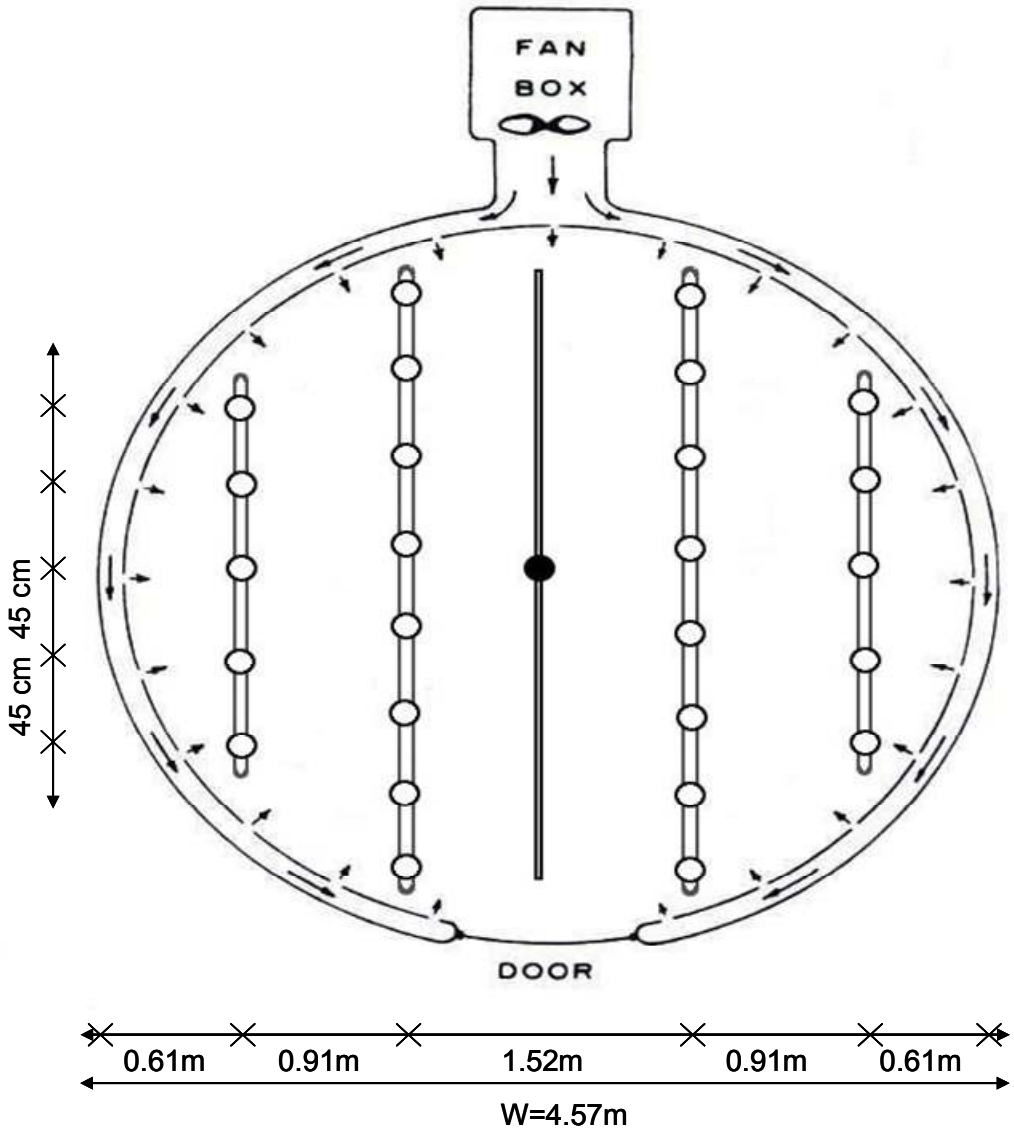


Figure 5. Light transmission at various wavelengths for a 0.02-cm (8-mil) thickness polyvinyl chloride plastic. Measurement was made with a Beckman DV-7 Spectrophotometer, Adapted from Heagle et al., 1989.

4.3 Plant Material and Growth Conditions

The soil was plowed twice before installing chambers and ambient plots. Before planting, soil was prepared by plowing each chamber area with a manual roto-tiller (rotatory cultivar). Three stems cuttings of the sugarcane variety US 67-22-2 were sown by hand on four furrows inside the chambers and ambient plots on December 7, 2005 (Appendix A-Figure A4). Cuttings were obtained from a previous experiment plot which had been sown for one year. Emergence started on December 15, 2005. The inner two furrows were 1.52 m (5 feet) apart from each other and the outer two furrows were 0.91 m (3 feet) apart from the inner ones (Figure 6). Three seedlings containing one node each were planted in each row at a distance of 0.45 m (1.5 feet). After emergence seedlings were thinned to one per seed. The plant density in each plot was 1.6 plant/m^2. A plastic mulch cover was used between furrows to control weeds. During the first four month manual weeding was used inside the furrows to ensure optimal crop growth. Plants grown on the sides of the perforated inner layer of the chamber where used to avoid possible edge effects**.** These plants were excluded from measurements**.** Additional sugarcane plants were sown outside each plot to prevent irradiation from the sides. A drip irrigation system consisting of four drip lines along the furrows were installed before planting on each chamber and ambient plots to supply all plants water needs. All plots received ambient rainfall and were irrigated to maintain soil water potentials close to field capacity.

A fertilizer (15-5-10) was applied to achieve 674 kg N/ ha to each of the plots in three equal applications at levels above common farming practice to prevent nutrient deficiency. First application was applied the day of sowing while the two others were applied 4 and 6 month later. Plants were sprayed twice (May 17 and May 25, 2006) with insecticide ASANA® XL (active ingredient esfenvalerate) at a rate of 0.01 kg a.i. /ha to control yellow sugarcane aphids (*Sipha flava*).



Sampling Positions

○ Sugarcane plants

● PAR sensor and temperature

— CO_2 sampling tube

Figure 6. Schematic diagram of sampling position for temperature, light intensity, carbon dioxide, and growth of sugarcane variety US-67-22-2. Air temperature and solar radiation were measure at a height of 1.4 m. CO_2 was measured at a height of 1.2 m. Center furrows were 1.52 m apart and edge furrows were 0.91 m apart from the center furrows.

4.4 Measurements

This experiment was conducted to determine the direct effect of chamber studies on sugarcane variety *S. spontaneum* cv. US-67-22-2. Two treatments were established chambered (CH) and ambient air plots (AA), with three replicates per treatment. Four main stem plants from the center furrows (two from each furrow) were randomly chosen from each plot and labeled to take measurements to the same plant during the studied period. The following measurements were taken: (a) plant height (from the ground to the highest ligule), (b) number of leaves, (c) number of tillers, (c) total leaf area, (d) leaf relative chlorophyll content, (e) leaf photosynthetic rate. Environmental monitoring included (a) air temperature, (b) solar radiation and (c) CO_2 concentration (Table 1).

Table 1. Physical and biological measurements taken on sugarcane variety US-67-22-2.

Physical Measurements	Physiological Measurements
Carbon dioxide (µmol/mol)	Plant Height (cm)
PAR (mol m^{-2} hr^{-1})	Total leaf area (cm^2)
Temperature (ºC)	Number of leaves
	Number of tillers
	Leaf Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)
	Leaf relative chlorophyll content

4.4.1 Physical Measurements

A shed was established next to the chambers and the research plot to accommodate all analytical instruments used to monitor weather atmospheric parameters inside and outside the chambers. Each chamber and ambient plots were equipped with the following set of instruments and sensors:

a. Quantum sensor (LI-190 SA, LI-COR, Lincoln, NE)
b. Copper –constantan thermocouples
c. An air tube to measure CO_2 concentration

Air samples at a height of 1.22 m (4 feet) above the ground were drawn continuously along the center of the chambers and ambient plots using polypropylene tubing (1/4 OD) and vacuum pumps (Model 2107CA Thomas Industries, Wisconsin, USA) (Appendix B-Figure B1 and B2). Small holes were punctured along the tubes 30 cm (1 feet) apart to allow uniform air sampling along the chamber. Air flowed at a rate of 20 L/min (LPM) approximately from the chambers and ambient plots was directed to separated glass sampling manifolds creating a uniform air mixture and also collecting water from condensation or precipitation (Figure 7). The sampling manifold consisted of six glass jars to which fittings where attached allowing the use of tubes connected to the solenoid valves and draining tubing for any water collected from rain events (Appendix B-Figure B3). Air passed from the manifold jars to a series of six solenoid valves (3-way, Brass Viton seal, ASCO, Hanover, NJ) trig by relays allowing a sequential activation causing the air to be sample independently from each plot, while other samples were exhausted.

Air drawn through the solenoids to the Infrared Gas Analyzer (IRGA) passed through flow meters (Dwyer Instruments, 1-10 LPM; Michigan City, Indiana) used to check the flow rate of the vacuum pumps and determine any problem in the pumps. Ambient CO_2 was constantly monitored using an IRGA LI-7000 (LI-COR Inc. Lincoln,

NE) (Appendix B-Figure B3). Before air reaching the analyzer a (T) fitting was used to exhaust excessive air and two miniature pumps (Model 3003-312 Thomas Industries, Wisconsin, USA) activated by a power supply were used to deliver 1 L/min (LPM) to the IRGA (Figure 7). The IRGA was set on an absolute mode using chemicals scrubbers which were change every two days. The chemicals were uses to purge CO_2 and water vapor from the ambient air continuously. This air was send to the reference cell in the IRGA and the air from the chambers was send to the sample cell. The IRGA calculated CO_2 concentration base on this difference in absorption of infrared radiation passing through the two gas sampling cells. At the beginning of the sampling period for each chamber, a delay of 15-s allowed for the IRGA and sample lines to be purged of previous gasses.

Solar radiation available to plants was studied by measuring photosynthetically active radiation (PAR) with a quantum sensor (LI-190SA, LI-COR Inc., Lincoln, NE). Temperature within chambers and ambient plots were determined using shaded copper-constantan thermocouples. Air temperature and photosynthetically active radiation (PAR) sensors were placed and continuously monitored at the center area on three chambers and two ambient plots at a height of 1.37 m (4.5 feet) above ground. Measurements for individual plots were completed in 30-s period and data was collected at 15-min intervals. This cycle and all data logging system functions were controlled and monitored using Edlog programming (Loggernet Campbell Scientific software) and a CR23X data acquisition system (Campbell Scientific, USA) connected to a desktop computer. Appendix C shows the CR23X program use to control the monitoring instruments.

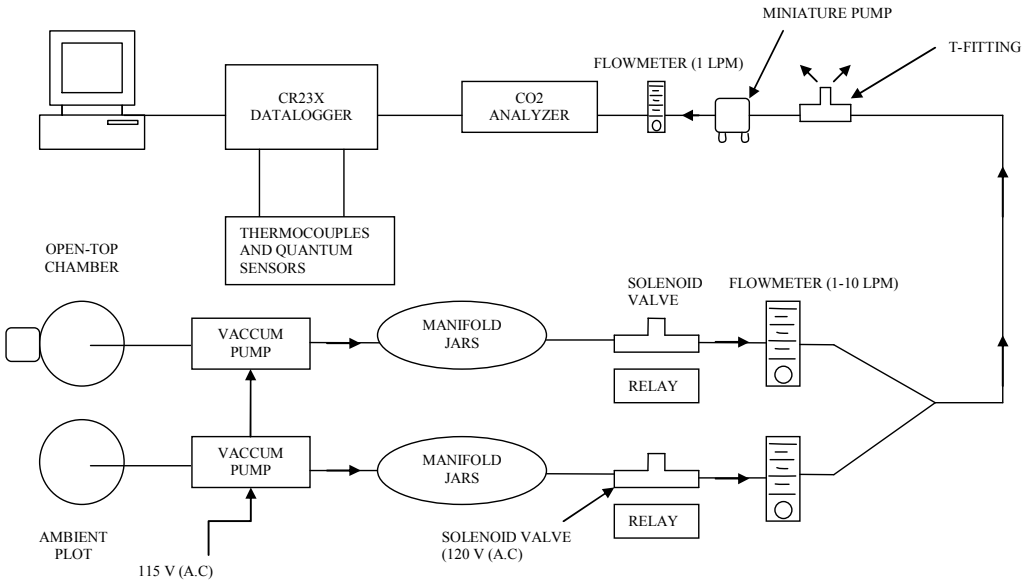


Figure 7. Schematic diagram of monitoring system

4.4.2 Physiological Measurements

Physiological measurements were taken throughout the experiment at regular intervals depending on the parameter. The following parameters were measured regularly: plant height, number of leaves, number of tillers, leaf photosynthesis and leaf chlorophyll content. Plant height (from the ground to the highest ligule), number of leaves, number of tillers and total leaf area measurements began 50 days after planting (DAP). Biweekly measurements were made during the first three month and monthly measurements after that. Measurements of leaf photosynthesis and leaf relative chlorophyll content were started at 140 (DAP).

Leaf gas exchange measurements were performed using a LI-6400 portable photosynthesis analyzer (LI-COR, Lincoln, NE). Measurements were made at the third means portion of the upper most fully developed leaf during the morning hours (9:00 am

to 11:00 am) (Figure 8). A light source with 1500 µmol m^{-2} s^{-1} photosynthetically active photon flux density was used as light source (6400-02 LED light source) for each measurement. External air was scrubbed of CO_2 and mixed with a supply of pure CO_2 using the 6400-01 CO_2 injector to result in a reference concentration of 400 µmol mol^{-1}. The flow rate of air through the chamber and sample IRGA was set to 500 µmol s^{-1} in order to minimize the system response time. Prior to every measurements calibration for the CO_2 mixer, calibration for the light source and matching of the IRGA'S were performed as recommended by the manufacturer (Li-COR 6400 Manual).

Total leaf area was measured with a CI-203 portable leaf area meter (CID, Inc. Camas, WA). Measurements were made on all leaves of the main shoot. Chlorophyll content was measured at the third mean portion of the upper most fully developed leaf once a month using a Field Scout CM 1000 Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, IL). Leaf relative chlorophyll content and the leaf photosynthesis were measured in the four marked plant of each plots using the main stem.

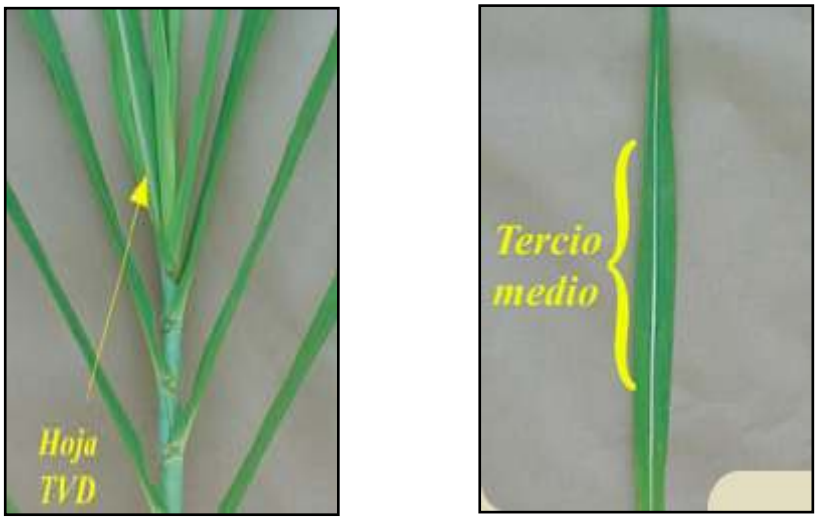


Figure 8. Portion of sugarcane leaf where photosynthesis was measured. Adapted from Cenicaña (2003).

4.5 Meteorological Data

Microclimate conditions in the open-field were observed from a weather station located 28 m from the experimental site. Data on air temperature, relative humidity, photosynthetic active radiation (PAR) and rainfall were monitored every minute but 60 – min average were recorded (Figure 9).

Figure 9. Weather Station by Campbell Scientific in the Lajas Agricultural Experimental Substation

4.6 Harvest

The sugarcane was harvested twice during the growth season at 300 and 360 DAP. Harvest consisted of removing all aerial parts of the plant that is available in one linear meter along the central inner furrow of the plot (Figure 10). Each harvest bunch was partitioned into green leaves, dried leaves, leaf apex and stem. Plants partitioned were place in separate paper bags to determine fresh weight. Dry weight was determined after drying to constant weight at 60ºC.

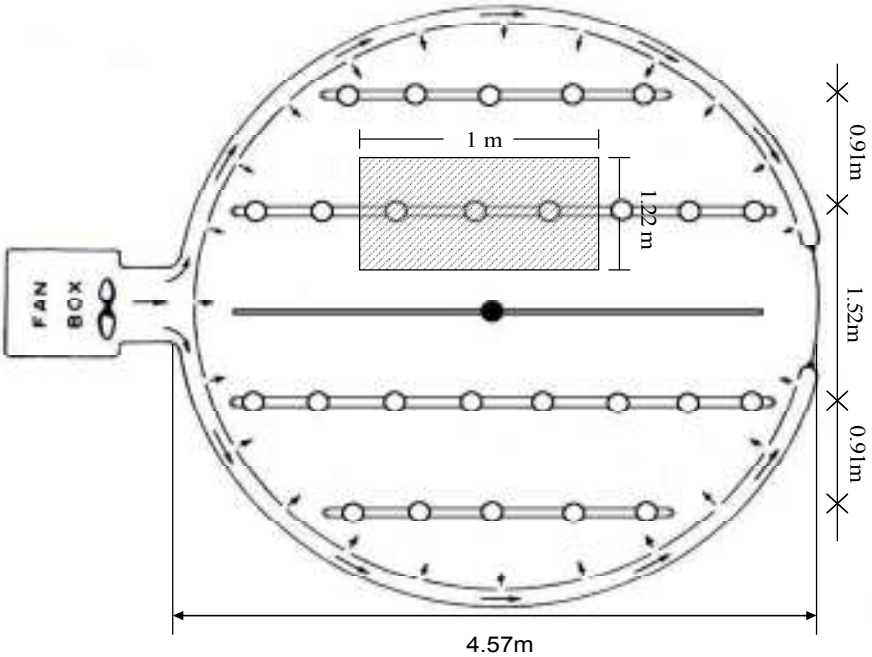


Figure 10. Schematic of harvest area

4.7 Experimental Design and Statistical Analysis

The experiment was conducted using a completely randomized design split over time with three replications. Chambers were the whole plots and each measurement (date) as the factor applied to the subplots. All data were checked for normality and homogeneity of variance and appropriate transformations where use to meet the requirements of the statistical tests. Only plant height data required square root transformation. For air temperature analysis, ten days were randomly selected from a subset of two categories according to daily mean ambient light intensity : < 20 moles m^{-2} day^{-1}(cloudy); > 50 moles m^{-2} day^{-1} (sunny). Data were analyzed as a split-plot design with type of day as the whole plot factor and treatments as the subplot factor. Physiological parameters were analyzed with an analysis of variance and trend analysis over time was conducted with orthogonal contrasts. Data on final biomass were analyzed as a split-plot design using treatments as whole plots factors and harvest as subplot factors. Statistical analysis were performed using Info-Stat v. 3.1 statistical software (InfoStat, 2006) with a significance level of $P<0.05$.

Best fitting curves were used to describe the relationship of height, number of leaves, number of tillers, and leaf area as explained by days after planting and thermal units expressed as degree days. All the data were used for the analysis of the variables. Statistical software Sigma Plot 10.0 was used for this analysis. The R^2 and the probability values of <0.05 were used as indicators of the efficacy of the model.

5. RESULTS AND DISCUSSION

5.1 Physical Measurements

5.1.1 Air Temperature

Air temperature during the daylight hours was generally higher inside the open-top chamber than outside. Air temperature within the open-top chamber averaged over 24 hours was 1.3 ºC higher than in the ambient plots. This value is within the range of temperature increases reported in previous experiments (Olszyk et al., 1980). Most studies have indicated a 1 to 3 ºC temperature rise within a chamber when compared to ambient (Heagle et al., 1989; Olszyk et al.1980).

However, short term differences of up to 2.5 ºC were also observed, especially on sunny days when temperature reached 37 ºC or higher. The average daily maximum temperature during the light period was 4.4 ºC higher inside the chamber than outside (38.3 ºC in CH versus 33.9 ºC in AA). Hendrey & Kimball (1994) measured a maximum increase of 6ºC for a chamber without plants with 4 air changes a minute. Adaros et al., (1989) found a maximum rise of air temperature in their open-top chamber to be 3.6 and 1.4 ºC under day and night conditions, respectively, but daily mean temperature increases were only by 1 ºC. Night temperatures in this research were found to be 0.9 to 1 ºC higher inside the chamber in comparison to ambient plot. Figure 11 shows the differences in maximum, minimum and average temperature between the two treatments. The higher temperature inside the CH resulted in a faster accumulation of degree days compared to AA plots (Figure 12).

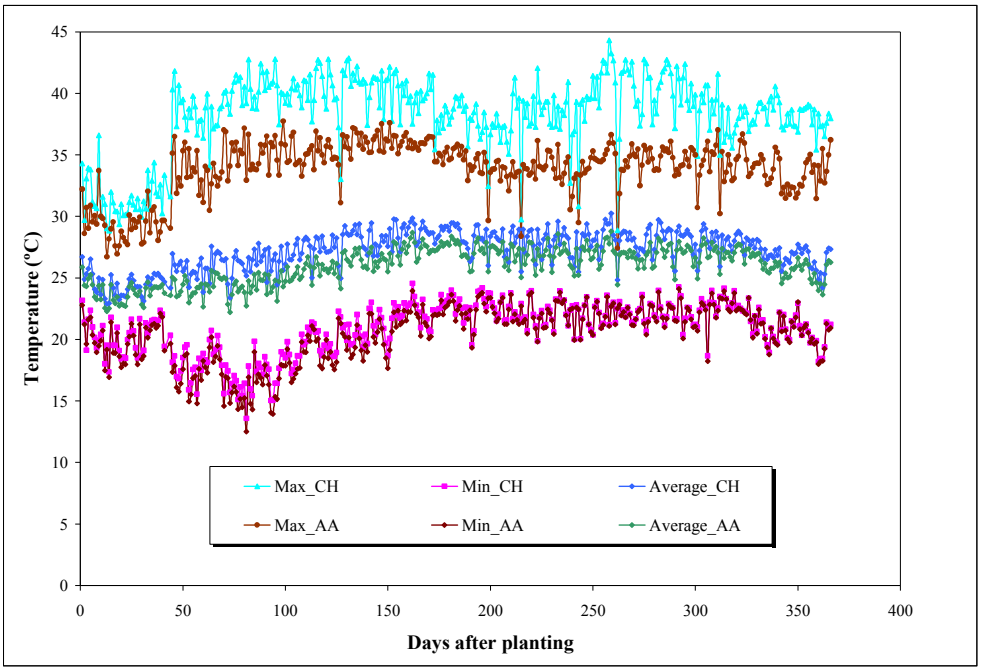


Figure 11. Daily maximum, minimum and average temperature between open- top chambers (CH) and ambient air plots (AA) from Dec. 2005 to Dec. 2006.

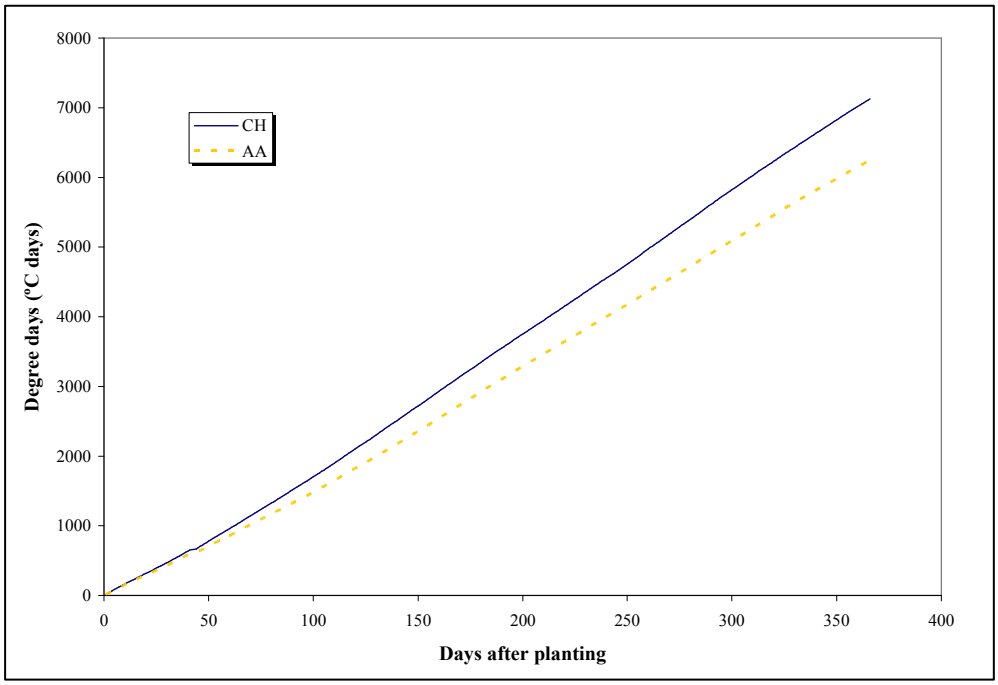


Figure 12. Accumulation of degree days in CH (——) and AA(-----) plots (base temperature of 10 ºC) from Dec. 2005 to Dec. 2006.

Figure 13 shows a typical daily performance of the open-top chambers and ambient air plot on a sunny day (February 4, 2006). Over this single day the variation in temperature was 1 to 6 ºC and the variation in light intensity was 400 to 1850 µmol $m^{-2}s^{-1}$ during daylight hours. Table 2 shows the differences between treatments on cloudy and sunny days. There was significant effect between treatments on cloudy days and sunny days on mean air temperature. On cloudy days a significant difference was found even though the difference was very small, 0.54 ºC higher in the chambers. On sunny days differences in temperatures between chamber and ambient plots were 1.8 ºC higher in CH than AA.

Temperature rise within the chamber is governed primarily by the solar energy absorbed, the rate of ventilation and the air flow regime within the chamber. A temperature rise will always exist within the chambers; a better controlled can be obtained when very high ventilation rates are employed. Only by the application of refrigeration can the temperature excess within the chamber be made negligible (Norris et al., 1996).

An important factor for a good chamber setup that involves CO_2 is to obtain airflow rates that will help to maintain temperature similar to the outside field. In this experiment the velocities of air exiting the holes in the duct were measured with a hotwire anemometer to verify the air exchange rate. Exit velocities of 8.6 to 11.08 m s^{-1} showed the air exchange rate to be approximately 3800 L s^{-1}(four air changes per minute).

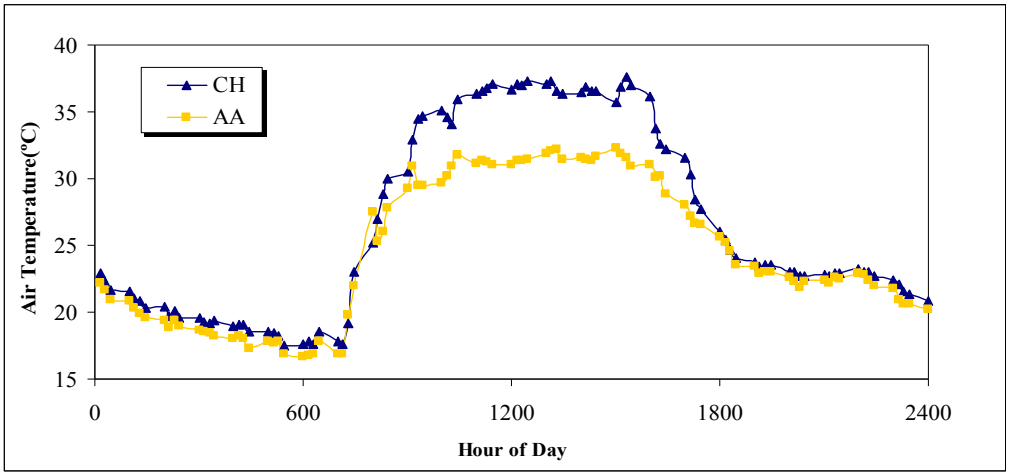


Figure 13. Diurnal variation of air temperature in the open-top chamber (▲) and the ambient plot (■) on February 4, 2006.

Table 2. Average air temperature (ºC) on cloudy and sunny days between open-top chambers and ambient plots.

	Type of Day	
Treatment	Cloudy	Sunny
CH	25.44 ± 0.20 at	28.85 ± 0.17 a
AA	24.90 ± 0.22 b	27.10 ± 0.24 b

t Each value is the average temperature ± SE of 10 days for each treatment and type of day. Means within a column followed by different letter are significantly different according to LSD Fisher test (0.05 level of confidence).

5.1.2 Light

Photosynthetically active radiation (PAR) energy was measured with a quantum sensor installed 1.2 m above ground surface at the center of the chamber and ambient plots. By April 17, 2006 (131 DAP) the plant canopy surpassed the sensor's elevation. On average PAR inside the chamber was generally 18-20% lower than the outside plots (Figure 14). This reduction could be attributed to the light transmission efficiency of the chamber walls, 8 mil PVC film, and the shading by the aluminum chamber framework. PAR in the open field was always higher than that in the chambers throughout the study period. The results favorably compared with those of Weigel et al. (1987) who reported a 18-20% reduction inside the chambers used that as a 3.15 m in diam; 2.40 m tall chamber but differs by Heagle et al. (1989) who reported a 15% less radiation with new plastics in same size chambers as we used. For example; during February 4, 2006 a clear sunny day at 12:00 a maximum PAR of 1458 µmol m^{-2} s^{-1} and 1817 µmol m^{-2} s^{-1} were observed inside and outside the chambers respectively (Figure 15). A relative difference of over 24% in PAR was available for plants.

In other experiments it has been found that these reductions are not uniform across the chamber surface and are influenced by the position of wall and structural supports (Mandl et al., 1989). In some areas, and for limited times, PAR levels were reported by Heagle et al.,(1979) to be greater than ambient at certain times during the sunny days because of reflection from the plastic walls. This effect by the polyvinyl chloride covers in some areas of the chamber was also noted by Weinstock et al. (1982) and Olszyk et al., (1980).

This effect was pointed out by Unsworth (1986) which showed a higher net radiation (20%) inside the chambers caused by an increase in long wave radiation. This is the caused of an increase of foliage temperatures inside the open-top chambers. The reflection from chambers walls was not measured in this study. Nor was it measured the reflection by the soil surface, although it should be low due to the dark color soil. These

components of PAR could have been measured using quantum sensors facing down and on top of the chamber or by the use of pyranometers. Measurements of solar radiation are important since the dry matter production of a plant canopy is directly proportional to the amount of PAR intercepted by the canopy.

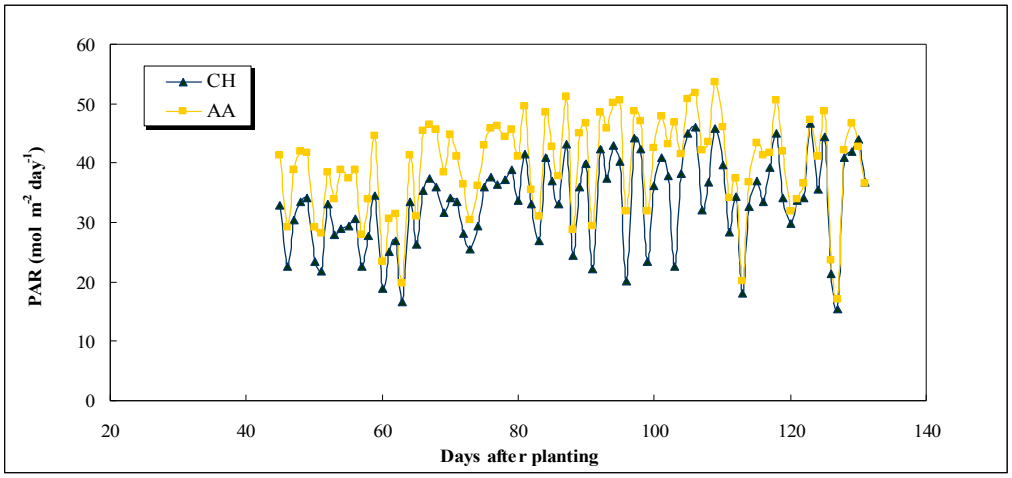


Figure 14. Daily PAR in the open-top chamber (▲) and in the ambient plot (■) from January to April 2006.

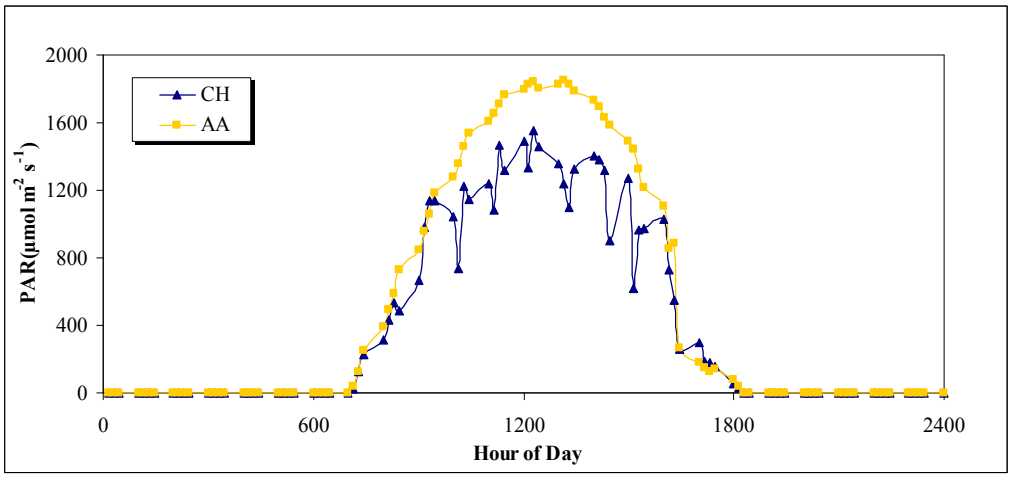


Figure 15. Diurnal variation in PAR 1.24 m above ground in the open-top chamber (▲) and in the ambient plot (■) on a sunny day (February 4, 2006).

5.1.3 CO_2 Environment

Carbon dioxide (CO_2) and energy absorbed as a fraction of PAR are required for photosynthesis to take place in the leaves of plants. CO_2 is the direct source of carbon for plant growth and development. Any increase in its concentration is expected to influence crop physiology and productivity. The two treatments showed almost identical CO_2 concentrations as measured at the chamber center. Figure 16 shows the hourly distribution pattern of CO_2 in the open–top chambers and in the ambient air plots for the entire growing period. At night CO_2 concentrations reached concentrations higher than 400 μmol mol^{-1} for the two treatments. During the day at 1200 hours concentrations where 365 μmol mol^{-1} and 368 μmol mol^{-1} inside and outside the chambers, respectively. CO_2 concentrations declined in the chambers and in the ambient plots during the daylight hours when sugarcane is mostly photosynthetically active. Figure 17 shows the diurnal distribution of CO_2 on a typical day (February 4, 2006). For this date, at 1200 h concentrations inside the chambers was 364 μmol mol^{-1} while in the outside field was 366 μmol mol^{-1}. This shows that the CO_2 source strength in the open-field did not differ from that inside the chambers. Allen et al. (1992) discussed field techniques for exposure of plants to CO_2 and mentions that ambient CO_2 levels and level inside chambers with no added CO_2 were virtually the same.

The plants are subdivided in 3 classes depending on the physical and chemical systems for utilizing CO_2. These are the C3, C4 and CAM plants. The plants in each group have different physical systems for utilizing CO_2. The C3 plants absorb CO_2 through the stomata on their leaves and use it immediately. In this plant the first product of carbon dioxide is a 3-carbon compound. This is the first stable product from the fixation of CO_2 in the Calvin Cycle. Once in the plant the carbon and oxygen are separated, the carbon is synthesized into a compound having 3 carbon molecules. The C4 plants have additional photosynthetic reactions in which the first detectable product resulting from CO_2 fixation is a four carbon compound. The C4 photosynthetic system has a modification for increasing CO_2 at the functional sites of the carboxylation enzyme,

RUBP carboxylase. Its uses PEP carboxylase systems which have a high affinity for CO_2 and are efficient at capturing it, even at low CO_2 concentration. The C4 plants have a supplementary method for CO_2 uptake which forms a C4 carbon molecule. The C4 group have specialized cells called "bundle sheath" cells that are use to temporarily store CO_2. After the CO_2 is captured by the PEP carboxylase system, malic acid is formed and quickly transported to the bundle sheath cells. Once there is decarboxylated by the enzyme and CO_2 is released. These cells cope in the sometime adverse climate allowing the plant to continue it's grow under adverse conditions. The C4 species such as corn and sugarcane are among the most highly productive crops. Although C4 plants have been categorized for not being good candidates for CO_2 enrichment, studies have proof that high CO_2 concentrations does produce higher yield in C4 plants.

At the University of Florida, Vu et al., (2006), grew sugarcane under field like conditions for sunlight in separated green houses with concentrations of 360 µmols mol^{-1} to 720 µmols mol^{-1}. At final harvest, leaf area of the ambient CO_2 plants was found to be higher in the elevated treatments. The elevated CO_2 increased the leaf area by over 30 percent. Similarly, the above ground mass of the plant increased from 602.0 grams per plant to 867.0 grams per plant (a 44% increase) in the elevated CO_2 treatment.

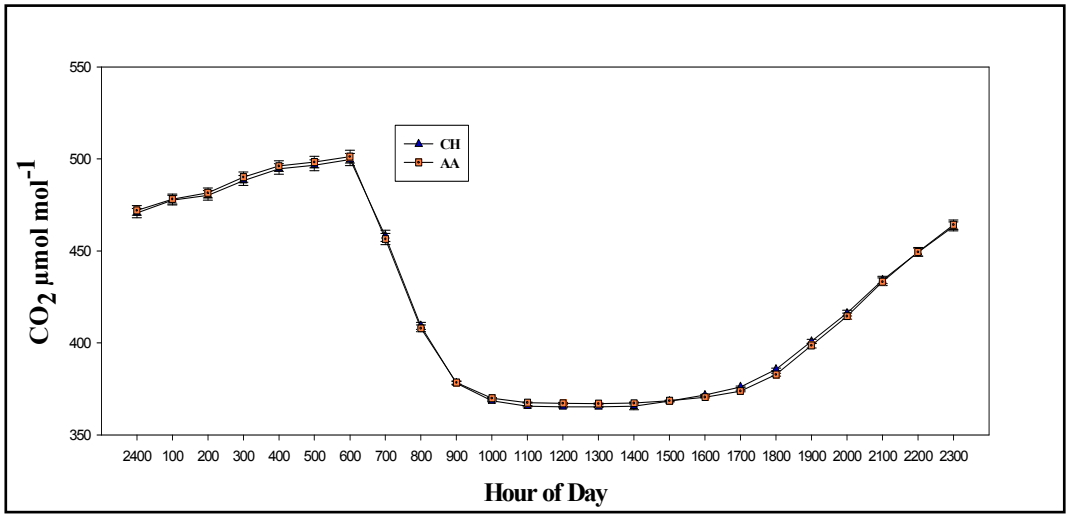


Figure 16. Hourly distribution for CO_2 in the open-top chamber (▲) and ambient plot (■) for the entire growing period.

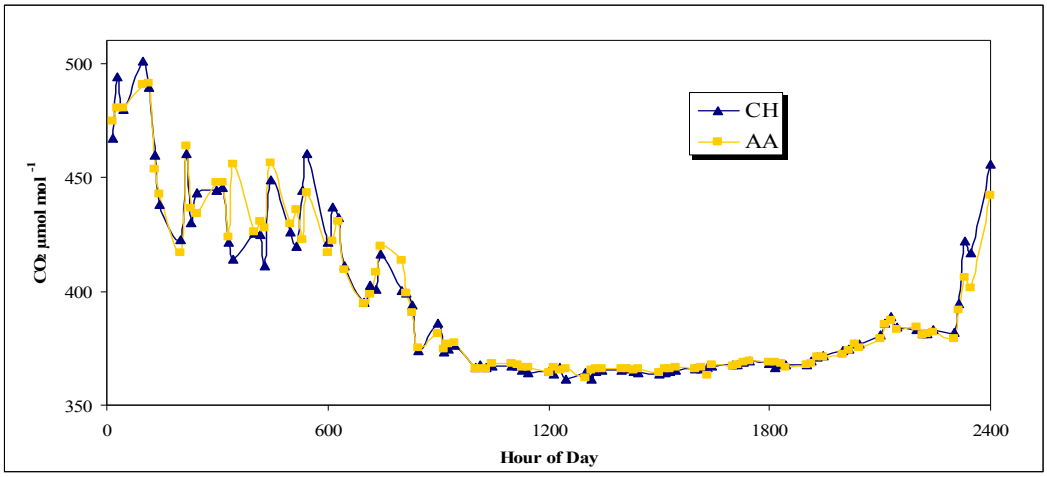


Figure 17. Diurnal variation in CO_2 in the open-top chamber (▲) and in the ambient plot (■) on February 4, 2006.

The higher night values of CO_2 may be explained by the process of plant respiration and the contributions from the soil. Organism living on and within the soil: beetle, worms, and other creatures, along with fungi, roots, bacteria and other microbes produce a constant flow of carbon dioxide as they respire. About a third of the gaseous carbon emitted from the soils comes from its uppermost layer of decomposing litter (Volk, 1994). As a consequence of respiration and microbial activity, the concentration of carbon dioxide is much higher in soil air than in the atmosphere. The flux of the carbon dioxide from the surface of the soil is an important component of the carbon budget of a prairie ecosystem that should be considered in studies with open-top chambers.

5.1.4 Microclimate in the open field

The average temperature in the open field for the growing period was 25 ºC. Daily temperatures ranged from a maximum of 37ºC on August 28 to a minimum of 13 ºC on February 26 (Table 3). Average relative humidity during the investigation was 79%. The total precipitation recorded was 1127 mm close to the historical 1162 mm mean annual precipitation observed at Lajas. The highest precipitation occurred on May with 171mm of rain. Table 3 shows monthly and average data during the studied period of the weather variables measured at Lajas Experimental Substation.

Table 3. Summary of weather parameters in the open field on 2006.

	Dec'05	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Period
Temperatures(˚C)													
Averages													
Daily Max	31	30	30	30	30	32	32	32	33	33	32	32	31
Daily Min	18	18	17	18	20	22	22	22	22	22	22	21	20
Monthly	24	23	23	23	25	26	27	27	27	26	27	26	25
Extremes													
Highest	32	31	32	31	32	36	33	35	37	34	34	36	37
Date	27	22	27	5	22	22	13	28	28	1	1	15	28-Aug
Lowest	15	16	13	15	18	18	19	19	20	20	18	19	13
Date	19	4	26	10	9	6	15	18	5	27	9	8	26-Feb
Relative Humidity(%)													
Average	78.3	77.0	78.9	78.78	79.89	77.32	*	79.38	79.7	80.35	83.0	*	79.3
PAR (mol/m^2 day)													
Average	31.97	32.86	37.74	41.06	42.03	43.88	41.03	38.99	36.09	38.83	35.30	36.06	37.99
Wind (m/s)													
Ave Max	3	3	3	3	3	4	4	3	3	3	3	3	3
Fastest	10	10	10	11	10	12	12	11	13	10	11	11	13
Date	10	21	26	24	13	12	13	23	10	5	7	28	10-Aug
Precipitation (mm)													
Total	7	75	45	162	110	171	88	95	91	78	97	108	1127
Greatest 24-hr	2.54	44	15	50	32	44	27	36	31	16	22	17	50
Date	30	7	16	16	25	28	23	25	26	12	27	17	16-Mar

* No Data available

5.2 Physiological Measurements

5.2.1 Plant Height

The plants in the open-top chambers grew taller than those on the ambient plots (Figure 18). Significant differences was observed during the experiment starting at 90 DAP. The figure show slow rates followed by a steep increase between 140 DAP and 240 DAP. The rate flattens out after 240 DAP. Over the studied period this difference in height increased was maintained until the end of the growing period. At 270 DAP plants grown in CH where 90 cm taller than that of AA plants. This represents a 34.4% increase in height in the CH.

This effect has also been reported for others crops grown inside chambers such as alfalfa (Olszyk et al., 1980) and wheat (Fuhrer et al., 1988). They concluded that the reduction in plant disturbance by air movement inside the chambers may have caused this increase in length. In the open field, plant height is reduced by the effect of mechanical shaking on internode elongation (Mitchell et al., 1975). Increase in air flow would increase plant shaking, and thus decrease internodes elongation and plant height (Mitchell et al., 1975). This effect seems to be a form of physiological stress rather than a physical damage. However inside an open-top chamber this limitation on stem elongation is removed, encouraging the growth of taller plants.

The wind in particular represents a form of mechanical stress which may have a powerful influence on the character that a plant develops in the natural environment. Mechanical stress effects on growth are known but not widely appreciated as an important force influencing plant development. Why species should differ in this way, and what is the endogenous control mechanism for mechanical stress, are unanswered questions. Brown and Leopold (1973) suggested that ethylene may serve as a natural stimulator of growth control associated with such physical stress as results from wind action. It had been suggested that plant growth response to physical contact, and that it represents and adaptation which protects plants from the stresses produce by winds. The

effect of phototropism (growth towards light) may also have induced the plant to grow taller caused by the production of plant hormones involved such as auxins. The auxins are involved in regulating the growing of plant shoots upwards.

It cannot be discarded that the higher temperature during the day and the faster accumulation of degree days may have influence on a higher height on sugarcane plants. Growing degree days accumulated more quickly inside the chambers, and increased the rate of development of sugarcane variety US-67-22-2. This difference in the development of sugarcane variety US-67-22-2 between the chambers and ambient plots can be explained by the difference in growing degree days accumulation.

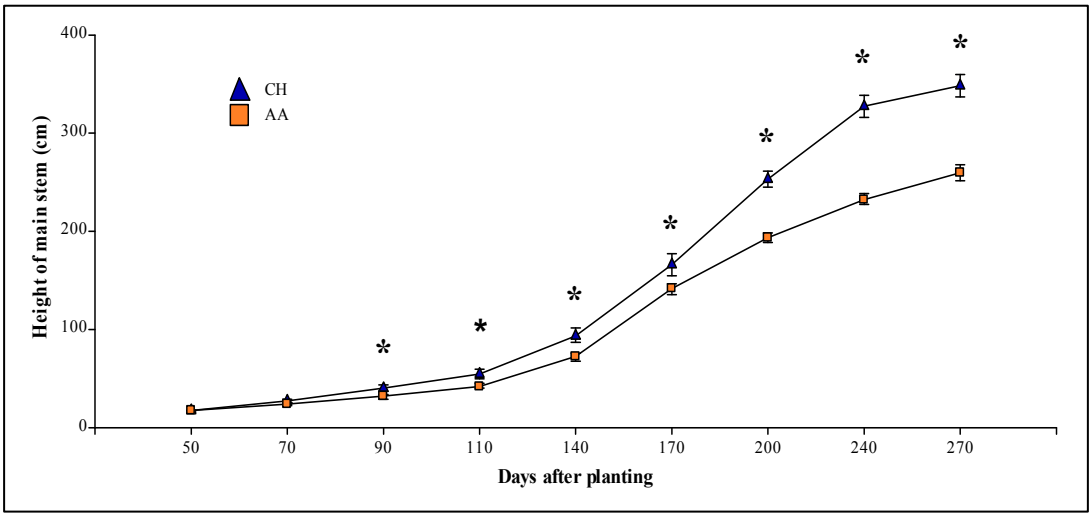


Figure 18. Height of main stem of sugarcane variety US-67-22-2 grown in CH (▲) and AA (■) plots. Bars represent standard error. * Significant at 0.05 level.

Plant height differences between CH and AA plots were negligible for the first few months when plotted against degree days accumulation (Figure 19). As DD accumulated by the higher temperatures inside the chambers, plants grew taller reaching 348.7 cm at 5185.9 DD thermal units. During the same growth period AA plants accumulated 4541.3 DD thermal units reaching 259 cm. Guindín-García (2003) observed a maximum plant height of 207cm with an application of 208 kg N/ha. Similar degree

days were observed during the studied period. Torres-Justiniano (2005) observed a plant height of 186 cm at 300 DAP with an application of 896 kg N /ha. These values differ to the ones observed in this investigation.

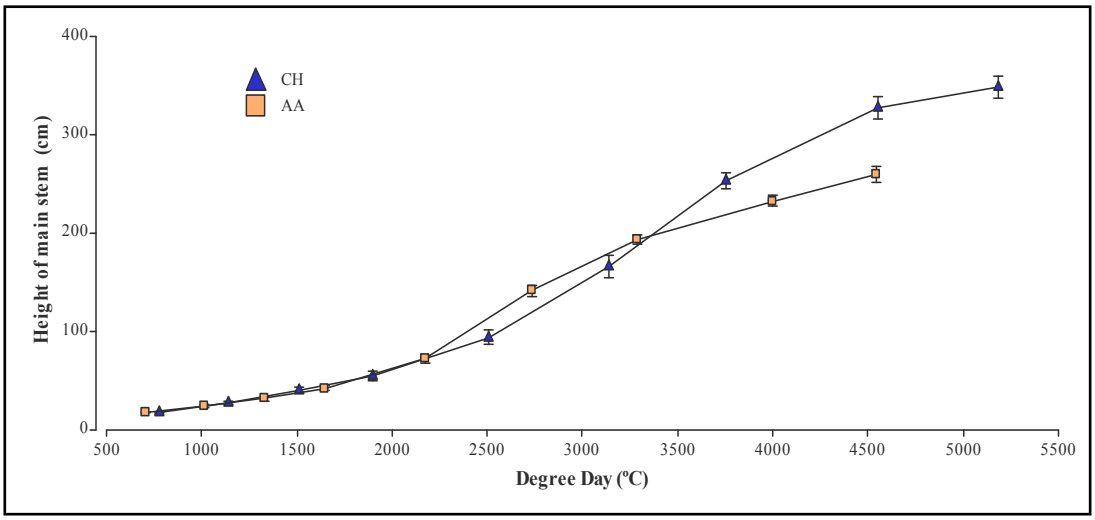


Figure 19. Height of main stem of sugarcane variety US-67-22-2 in terms of degree days (º C-day) from January to September 2006.

5.2.2 Number of Leaves

Significant differences in number of leaves were observed between treatments starting at 140 DAP. These differences were maintained until the end of the growing period (Figure 20). The highest number of leaves was observed at 170 DAP where the CH treatment had 13 leaves and AA treatment had 12 leaves. A decline on the number of leaves was observed at 200 DAP which continued until the end of the growing period, by this time the number of leaves in the CH had 10 leaves while the AA treatment had 9 leaves. Torres-Justiniano, (2005) reported 12 leaves at 270 DAP with a level of 896 kg N/ha. The same amount of leaves was observed by Guindín-García (2003). Alexander (1985) reported that energy cane encourages large stems with extended viability of leaves

(10 to 15 viable leaf ranks) compare to other sugarcane variety with (6-8 viable leaf ranks).

This decline in the number of leaves may have been cause by the distance of 0.91 m left between the outer and inner furrows. Alexander (1985) recommends row spacing between 1.4 to 2 m (4.5 to 7.0 feet) for energy cane variety US-67-22-2. Spacing's between 1.5 to 1.8 m (5.0 to 6.0 feet) appear suitable for most first-generation varieties, but wider spacing's have distinct advantages when yield are not significantly reduced (Alexander et al.,1982). The high plant density in the canopy could have restricted light penetration making the lower leaves to senescence faster. Row spacing and less plant density for energy cane should be considered in future research.

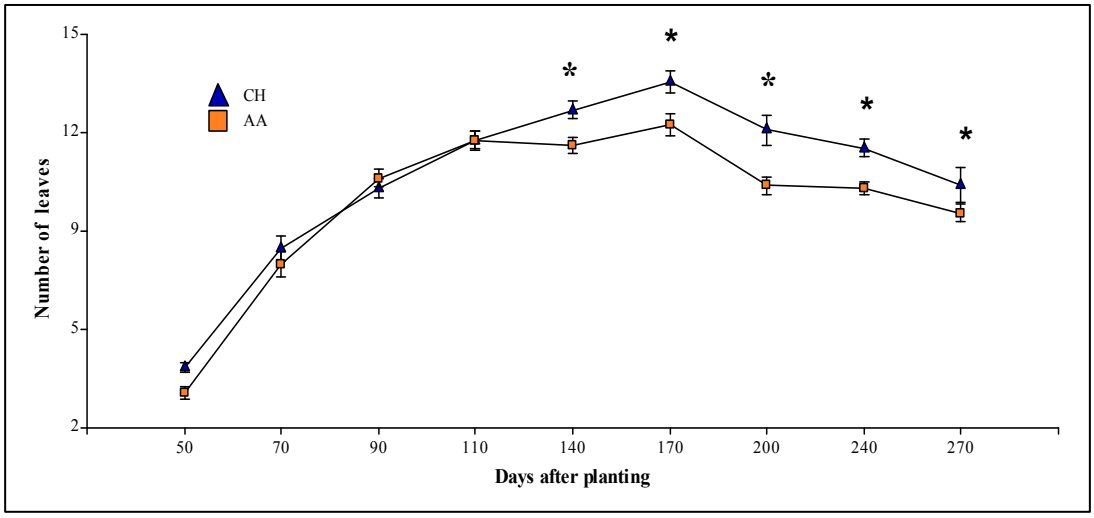


Figure 20. Number of leaves of main stem of sugarcane variety US-67-22-2 grown in CH (▲) and AA (■). Bars represent standard error.* Significant at 0.05 level.

When the number of leaves where plotted against degree day accumulation for the first few points an interaction was observed between treatments when at 3000 DD a faster development of leaves where seen in the chamber treatments (Figure 21). A maximum of 13 leaves was reached inside the chambers at 3140 degree days and 12 leaves at 2733 degree days for the ambient plot. A decrease in number of leaves was observed at 3751

degree days for the chamber plots and at 3287 for the ambient plot. This decrease was maintained until the end of the studied period.

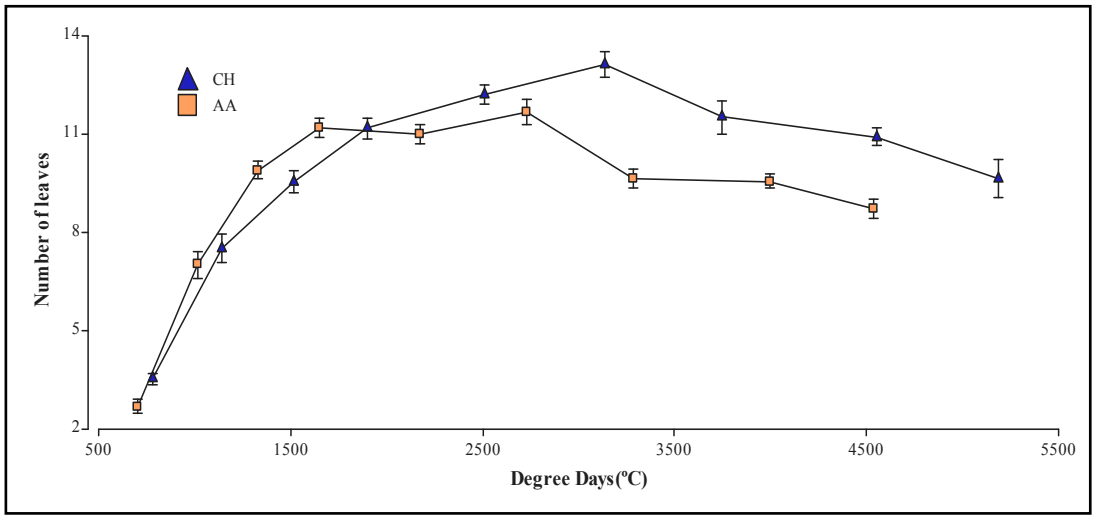


Figure 21. Number of leaves of main stem of sugarcane US-67-22-2 in terms of Degree Days (ºC-day) from January to September 2006.

5.2.3 Number of Tillers

Tiller density on sugarcane is a dynamic variable and part of the canopy that interacts with the environment. The US-67-22-2 variety is characterized for high tillering. Significant difference were started to be seen at 200 DAP and until the end of the growing period (Figure 22). The number of tillers were started to be seen at 70 DAP for both treatments with an average of 3 tillers for both treatments. A maximum of 21 tillers was observed at 170 DAP for the chamber treatment and 19 tillers in the ambient plot treatment. On average for the entire growing period the chamber treatment developed 11 tillers while the ambient plot developed 10 tillers. At 200 DAP a decline was observed which continue along the end of the growing period where it stabilize at 11 and 9 tillers for the CH and AA treatments, respectively. Torres-Justiniano (2005) observed similar number of tillers for this same variety sugarcane although the behavior of the variable

was of an exponential increment. The decline at 200 DAP could be explained by the light competition that tillers passed during its life cycle.

The typical life cycle of an individual tiller of sugarcane has been well described by (Van Dillewijn 1952, cited by Bezuidenhout et al., 2003). Initially the bud remains dormant. Then, after germination, the new shoot will elongate towards the surface of the soil and once it has emerged, is known as a primary tiller. After emergence, leaves will develop and the primary tiller will start tillering. The tillering process produces a stool of upright stalks containing one primary tiller and various numbers of higher order secondary and tertiary tillers. The continuing developed tillers will be competing and under severe light competitions in the later stages of the crop will eventually undergo senescence. Van Dillewijn (1952) identified light intensity and day length as the most important driving factors for tillering, while temperature was consider to be the second most important factor.

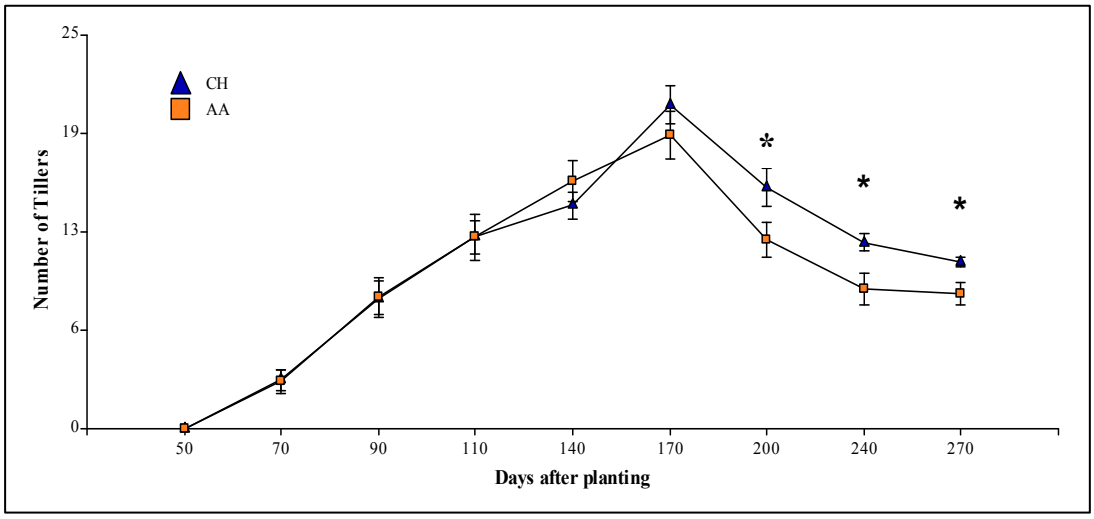


Figure 22. Number of tillers for CH (▲) and AA plot (■). * Significant at 0.05 level.

In terms of degree day, tillers followed a similar trend as the number of leaves. At the beginning of the experiment no difference was found between treatments when compared against degree days. A maximum number of tillers of 21 were reached at 3140 degree days for the chamber treatment and 19 at 2733 for the ambient treatment. A decline was observed for both treatments starting at 3751 degree days for the chamber treatment and at 3287 degree days for the ambient treatment (Figure 23).

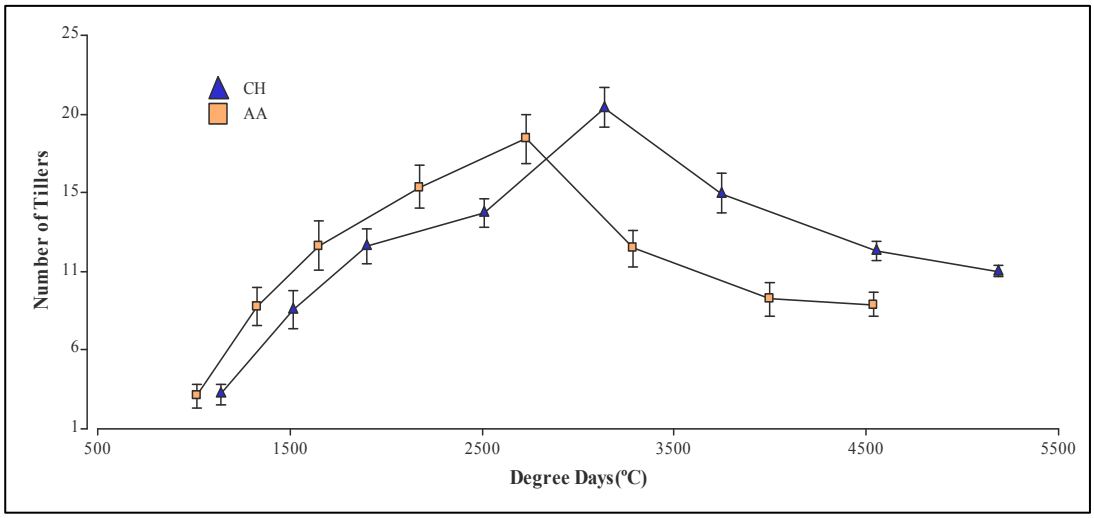


Figure 23. Number of tillers of sugarcane variety US-67-22-2 in terms of Degree Day (ºC-day) from January to September 2006.

5.2.4 Total Leaf Area

In general total leaf area inside the chamber was 20% higher than the ambient air plots (6208.3 cm^2 CH versus 5144.0 cm^2 AA) with significant difference. A significant interaction was found between the treatments and time (Figure 24). At 90 DAP a difference of 30% more leaf area inside the chambers was observed compared to the ambient plots. At 140 DAP a 15 % higher was found in the chamber compare to the ambient plots. Combining the data for 200 DAP and 240 DAP it can be seen that in both dates leaf area was generally 20% higher inside the chambers when compare to the ambient plots. This increase in leaf area was apparently the result of an increase in the number of leaves as well the size of the leaves inside the chamber treatment. Leaf area development may have also been influenced by the increased in temperature inside the chambers and a faster accumulation of degree days. There may have been an increase in the rate of expansion, contributing to the observed larger leaf area of the chamber plot.

Sanders et al., (1991) found that chamber grown plants of bean (*Vicia faba*) developed larger leaf area than those in the ambient plots. This difference was attributed to the effect of the chamber on air temperature and radiation since these are important in determining the final size of *V. faba* leaves. Kasim and Dennet (1986) showed that a 34% reduction in radiation was sufficient to induce a 23 % increase in leaf area for *V. faba*. The PAR reduction of 18 to 20% may have been sufficient to induce a partial shade response on sugarcane, contributing to the observed increase in leaf area.

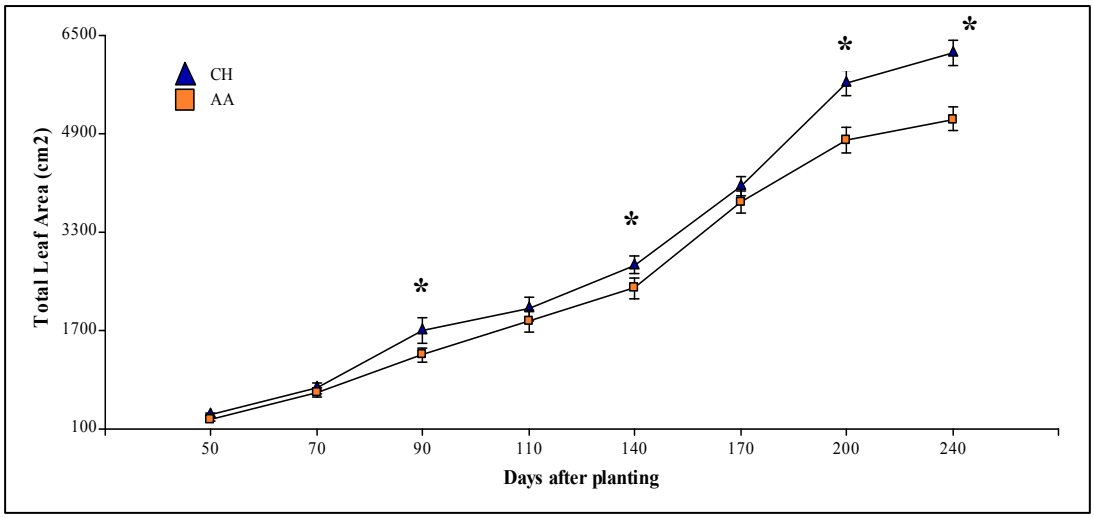


Figure 24. Total leaf area of main stem of CH (▲) and AA (■) plots. * Significant at 0.05 level.

In terms of degree days accumulation, at 4555 degree days, leaf area inside the chambers was 6208 cm^2 while in the ambient plots at 4001 degree days, leaf area was 5144 cm^2 (Figure 25). At the beginning of experiment no differences is seen between leaf area although at the end of the growing season a difference was observed showing an increase of leaf area inside the chambers.

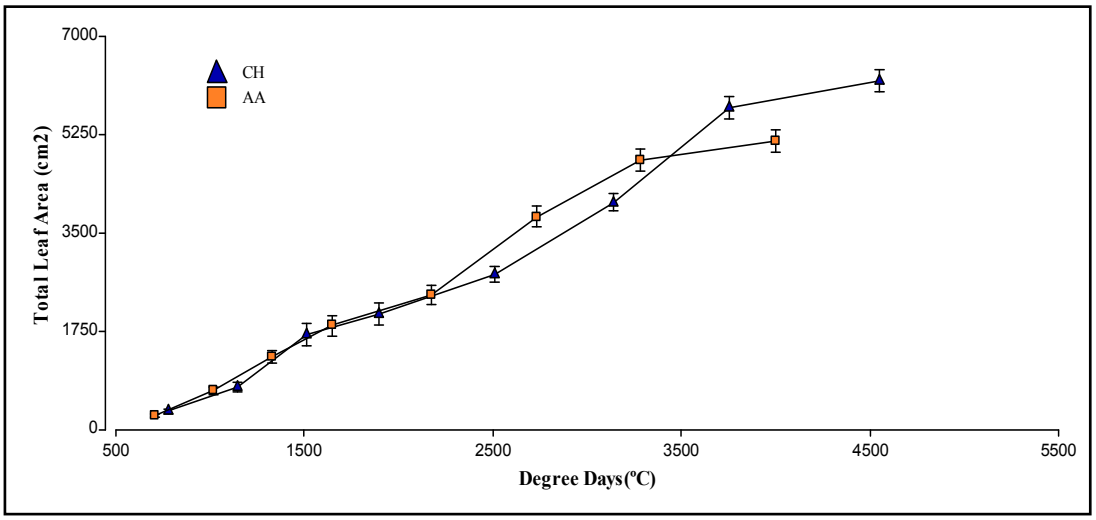


Figure 25. Total leaf area of main stem in terms of degree days (ºC-day)

5.2.5 Leaf photosynthesis

Photosynthesis was measured at the third mean portion of the upper most fully developed leaf. Figure 26 shows the values of photosynthesis for the two treatments at different times after planting. The mean value for the chamber and ambient plot was relatively stable with no significant difference between treatments. The average assimilation rate for the growing period was 29 µmol CO_2 m^{-2} s^{-1} for the chamber treatment and 26 µmol CO_2 m^{-2} s^{-1} for the ambient treatment. Sanchez-Espino et al., 1999 found no difference in photosynthesis in maize grown inside open-top chambers and the exterior control. Oijen et al., 1999 studied the interaction of temperature and CO_2 of spring wheat grown in open-top chambers. He found that measurements in the ambient plots showed equal or decrease photosynthetic rate compared to cooled open-top chambers. No significant interaction between CO_2 and temperature was found on leaf photosynthesis in spring wheat. Schenone et al. (1994) observed a decline in net photosynthesis toward the end of august in bean plants for both filtered chambers and external plots, although it was attributed to the effect of air pollution, especially ozone.

In this investigation a decline was observed for both treatments at 240 DAP which continue until 260 DAP. At the last sampling date a rise was observed in the chamber treatment which was significant compare to the ambient plot. This decline may have been due to a reduction in solar radiation during that period of time. The graph shows the mean values of PAR from the weather station during the period photosynthesis measurements were made. A decline is seen for those periods of measurements. Although a combination of factors may have contributed to this decline. A possible slightly under-irrigation could have caused a water stress. Water is essential to maintain cell and leaf turgor. Any moisture deficiencies to the plant result in dehydration of cells and leaves wilting. These effects slow the rate of photosynthesis.

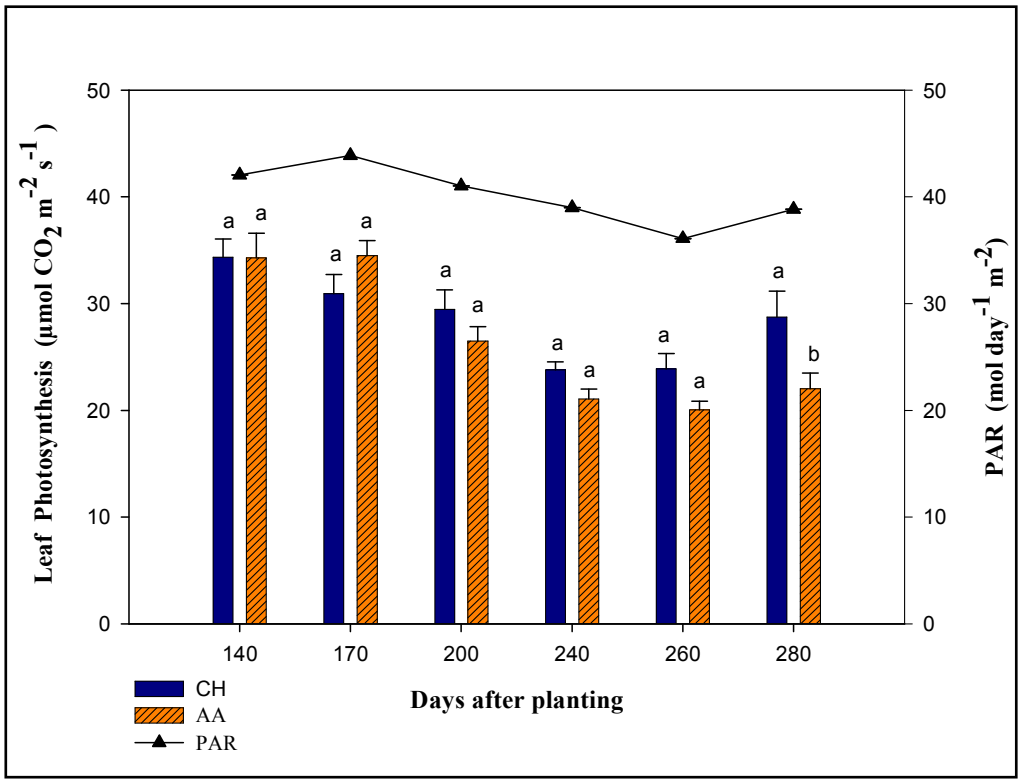


Figure 26. Leaf photosynthesis in chamber and ambient plots with PAR from weather station. Bars represent standard error. Different letters within a day are significantly different from each other (P<0.05).

5.2.6 Leaf relative chlorophyll content

Leaf relative chlorophyll content was measured at the third mean portion of the upper most fully developed leaf. The Field Scout CM1000 Chlorophyll Meter determines relative chlorophyll content index through measurement of two 'light' wavelengths - 700 nm (red) and 840 nm (near-infrared). The device senses these wavelengths from the light source and those reflected from the targeted surface. Since chlorophyll absorbs red light (700 nm) and reflects near-infrared (840 nm), the instrument compares the results of these measurements and calculates an estimate of chlorophyll content (range 0 to 999) (Spectrum Tech. CM 1000 Manual). Refer to Torres Justiniano (2005) for calibration graph.

Figure 27 shows the values of chlorophyll content for the two treatments at different times after planting. In general chlorophyll content was constant during the growing period with no significant difference between treatments. Only at 200 DAP a significant increase in chlorophyll content was observed in the ambient plot. Olszyk et al., 1992 found that across five evaluations of total chlorophyll content on orange trees, only in April 1987 was significantly higher in the non-filtered chambers compare to the chambers. This result differs from Janous et al., 1996 which found a continual increase of chlorophyll content during the cultivation of Norway spruce trees grown in open-top chambers in comparison to the control.

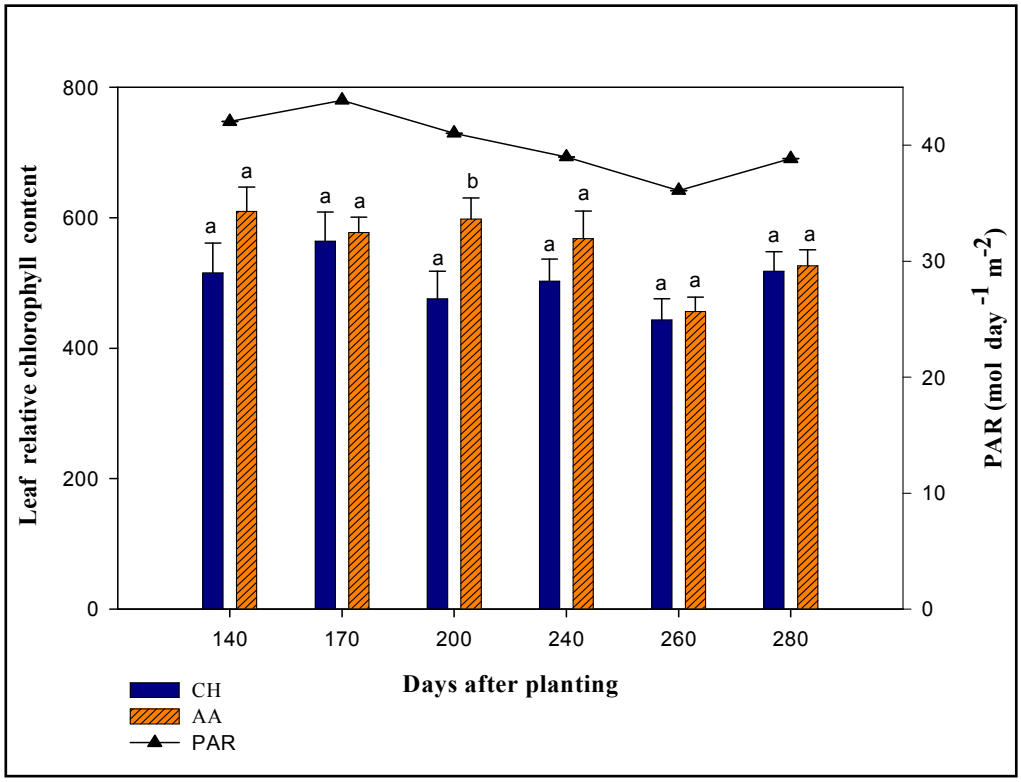


Figure 27. Leaf chlorophyll content for chamber and ambient plot with PAR from weather station. Bars represent standard errors. Different letters within a day are significantly different from each other (P<0.05).

5.2.7 Sugarcane Aboveground Biomass

Table 4 shows the aboveground biomass for both harvest dates and partitioned parts of the sugarcane. At the first harvest date the total dried aboveground biomass produced by plants in the open-field was 7.68 kg/m^2 compared to 6.05 kg/m^2 by plants in the open-top chambers. This represents a 27% more aboveground biomass in the ambient plots than in the chambers. In the second harvest a similar behavior was observed but this time with a 45% more aboveground biomass in the ambient plots. Overall, plants in the open-field produced on average 37% more aboveground biomass than plants in the chamber treatment. These differences were not statistically significant. There was no significant difference in any of the fresh and dried weight components between open-top chambers and ambient air plots. Biomass in the separate plots varied greatly resulting in large standard deviation and consequently reducing the precision of these comparisons.

This results show similar response with other researchers as Olszyk et al., (1980) which found no significant effect on alfalfa yield although the plants were observed to grow taller. Howell et al. (1979) reported that yield of plants inside the chambers were sometimes greater than and sometimes less than yield of plants grown outside. Heagle et al. 1979 and Heggestad et al., (1980) found a number of crops that grow taller in the open-top chamber than in the open-field, without marked effect on yield. Olszyk et al., 1980 came to a conclusion, that differences existed between growth statistics inside and outside the chambers, but they tended to be random rather than systematic. This led the authors to the conclusions that such differences were not directly attributed to the effects of the chambers on physiological and microclimate parameters.

Table 4. Sugarcane US-67-22-2 aboveground biomass in open-top chambers (CH) and ambient air plots (AA) for both harvest dates. [a]

Parameter (kg/m^2)[b]	Treatment	Date of harvest 2006 11-Oct	8-Dic
Stem fresh weight	CH	19.81 a	25.36 a
	AA	18.75 a	26.85 a
Stem dry weight	CH	3.63 a	4.86 a
	AA	4.72 a	7.14 a
Leaf apex fresh weight	CH	3.19 a	3.24 a
	AA	3.76 a	4.71 a
Leaf apex dry weight	CH	0.82 a	0.81 a
	AA	1.06 a	1.49 a
Green leaves fresh weight	CH	2.13 a	1.39 a
	AA	2.48 a	1.48 a
Green leaves dry weight	CH	0.84 a	0.48 a
	AA	0.89 a	0.60 a
Dead leaves fresh weight	CH	0.92 a	1.15 a
	AA	1.33 a	1.33 a
Dead leaves dry weight	CH	0.76 a	0.95 a
	AA	1.01 a	1.13 a
Total fresh weight	CH	26.04 a	31.14 a
	AA	26.32 a	34.37 a
Total dry weight	CH	6.05 a	7.11 a
	AA	7.68 a	10.35 a

[a] Results are mean values from three chambers and three ambient air plots. Harvest weights in a column followed by different letters are significantly different.

[b] $1 kg/m^2 = 10$ ton/ha

5.3 Statistical models for physiological variables predictions on sugarcane variety US-67-22-2.

The statistical models were developed to be used as tools of prediction for the crop behavior based on climatologic conditions. This is considered an important tool that could be used in any part of the world. The statistical models that best fitted the physiological variables of height, leaf area, number of leaves and number of tillers where the sigmoid and quadratic model, respectively. This models where correlated with days after planting and degree days. In this study the variables presented values of R^2 similar for the regressions made with days after planting and degree days.

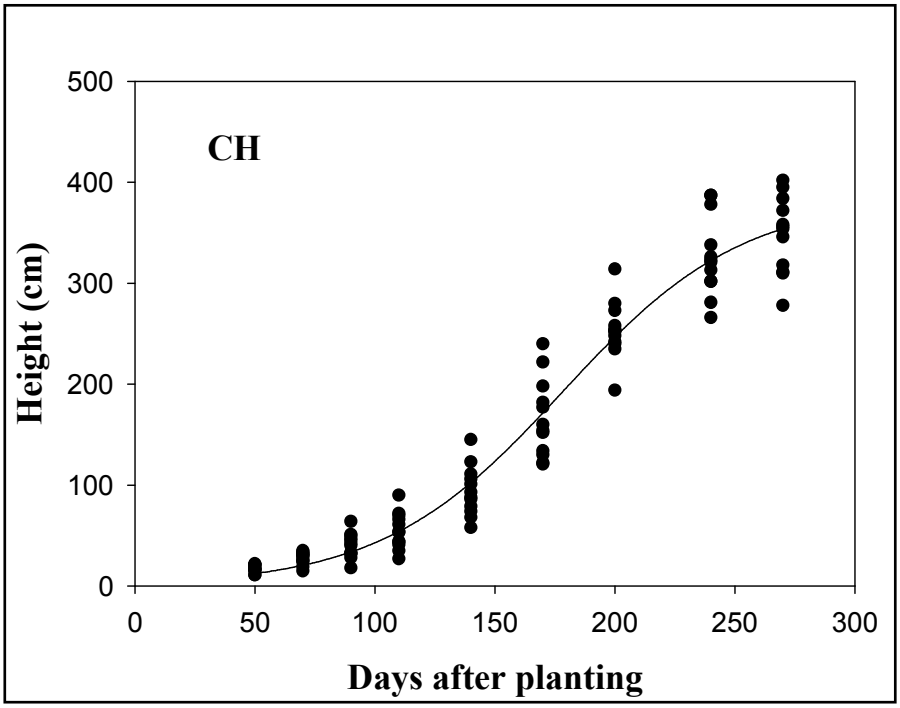
5.3.1 Plant Height

The model that best fitted plant height was the sigmoid model. This model allowed a good representation of plant height which shows a slow growth behavior at the initial stage of the cycle, fallowed by a rapid growth stage. As a function of DAP and DD in the chamber treatment a coefficient of determination (R^2) of 0.96 was obtained with a statistical significant probability value of ($p<0.0001$). For the ambient plot treatment a coefficient of determination of (R^2) of 0.97 was obtained for both DAP and DD (Table 5). The increase in plant height is the most obvious change in growth of most plants. The pattern of increase in plant height with age may be very similar to the dry weight increase in some species, but not in others. Guindín-García (2003) simulated plant height with respect to days after planting using the CANEGRO model. The model presented a relatively steeper growth starting at 114 days after planting. This is similar to the increases of plant height observed in our research which is seen to become larger after 110 days after planting (Figure 28 and Figure 29). The slow growth seen in our experiment at the beginning of the season may be attributed to the relative small leaf area available for light interception and therefore photosynthesis.

Table 5. Predicting models for sugarcane main stem plant height

Model Equation	Treatment	Predictor	R^2	P-value
H= 384.98/(1+exp(-(DAP-178.24)/37.67))	CH	DAP	0.96	<0.0001
H= 384.47/(1+exp(-(DD-3299.87)/767.02))	CH	DD	0.96	<0.0001
H= 276.64/(1+exp(-(DAP-171.35)/37.66))	AA	DAP	0.97	<0.0001
H= 274.93/(1+exp(-(DD-2747.41)/670.14))	AA	DD	0.97	<0.0001

CH- chamber treatment, AA- ambient plot, DAP-days after planting, DD-degree days
H- height of main stem



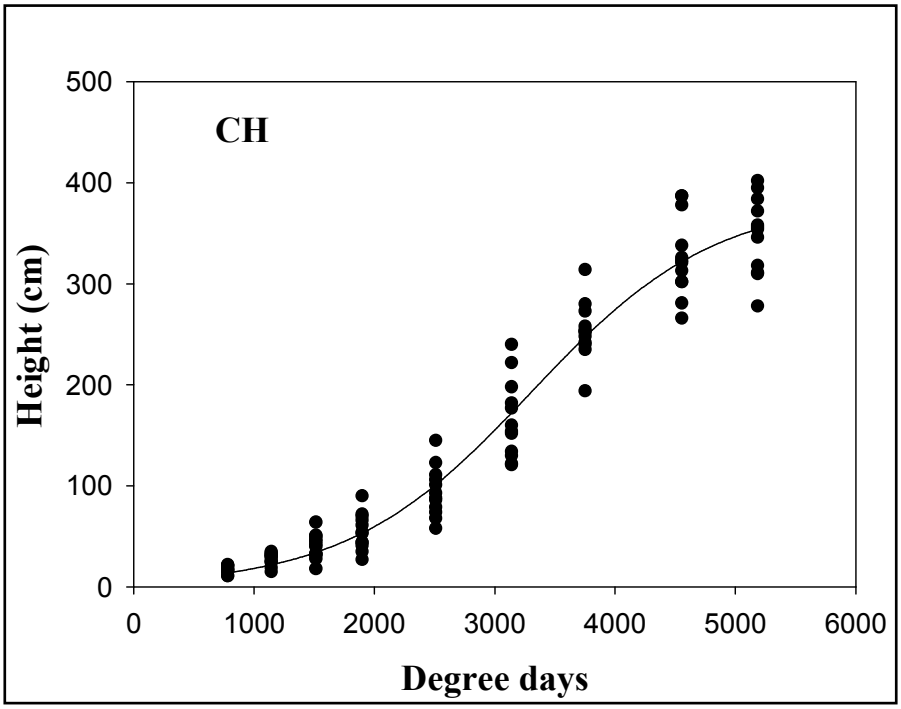


Figure 28. Sigmoid model graphs of height for chamber treatment (CH) with days after planting (DAP) and degree days (DD).

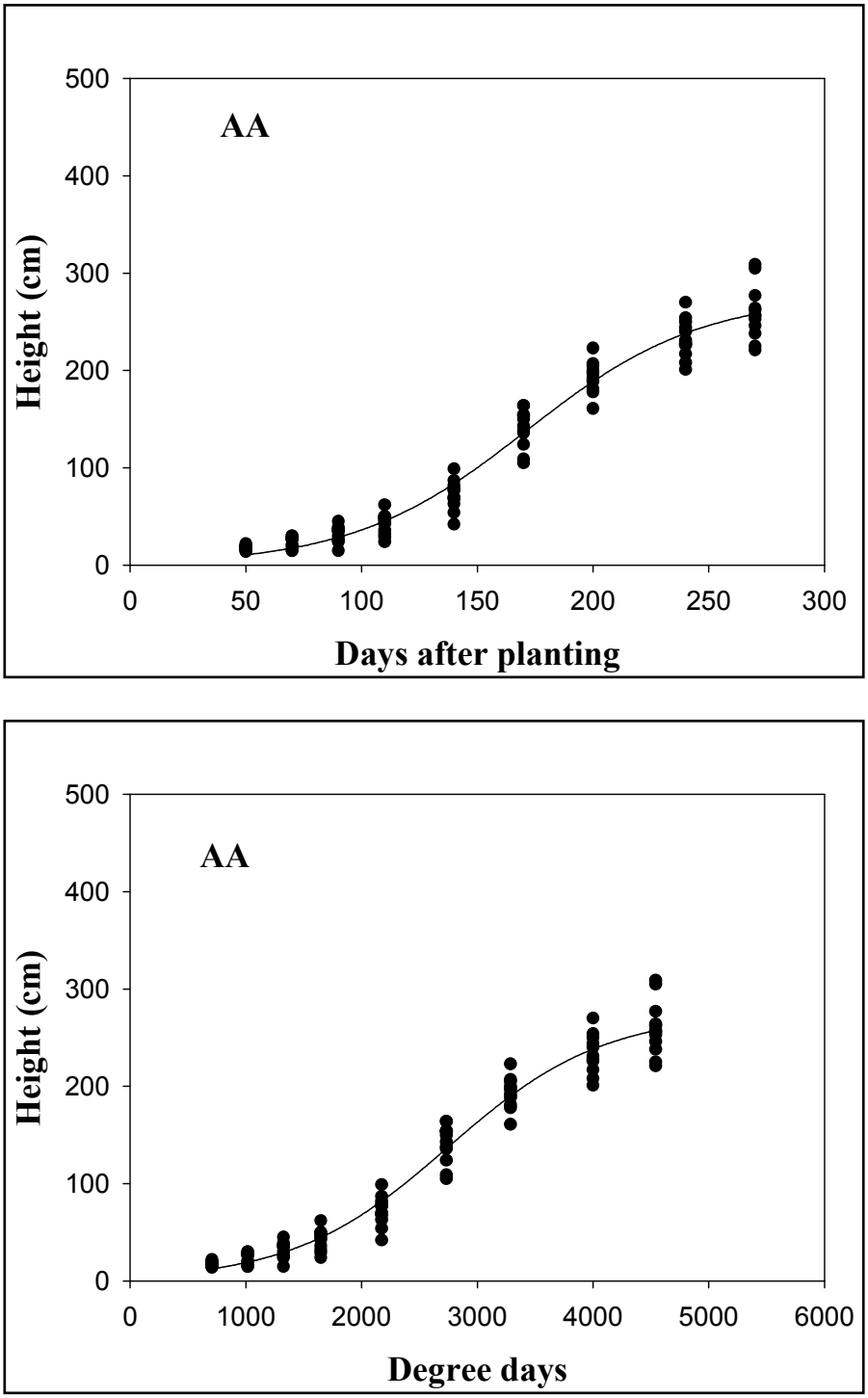


Figure 29. Sigmoid model graphs of height for ambient treatment (AA) with days after planting (DAP) and degree days (DD).

5.3.2 Total Leaf Area

The sigmoid curve was selected in this investigation for fitting the leaf area data. The coefficient values show that the model fitted well the data, with an R^2 of 0.92 for the chamber treatment and a R^2 of 0.91 for the ambient air plot treatment. The linear model was also evaluated resulting in a good statistical fit of the experimental data; however, it does not provide a biological explanation for the crop's development. Both treatments were statistical significant (Table 6). Leaf area is important, because it determines the rates of increase in the photosynthetic capacity of the plant. The changes in leaf area showed a sigmoid behavior, they increase gradually beginning at 90 DAP followed by a steep increment at 140 DAP and then at 240 DAP starts to stabilize (Figure 30 and Figure 31). This process coincided with the stabilization of height starting to be seen at 240 DAP.

Torres-Justiniano (2005), observed a similar stabilization of US-67-22-2 at 240 DAP for the 849 kg N/ha treatment where leaf area started to stabilize. The zero nitrogen treatment showed a linear trend showing a low increment of leaf area when compare to the other nitrogen treatments.

Table 6. Predicting models for leaf area of main stem

Model Equation	Treatment	Predictor	R^2	P-value
LA= 7188.74/(1+exp(-(DAP-154.01)/42.71))	CH	DAP	0.92	<0.0001
LA= 7140.03/(1+exp(-(DD-2795.02)/855.90))	CH	DD	0.92	<0.0001
LA= 5660.45/(1+exp(-(DAP-143.55)/38.19))	AA	DAP	0.91	<0.0001
LA= 5567.85/(1+exp(-(DD-2232.44)/654.20))	AA	DD	0.91	<0.0001

CH- chamber treatment, AA- ambient plot, DAP-days after planting, DD-degree days
LA- total leaf area

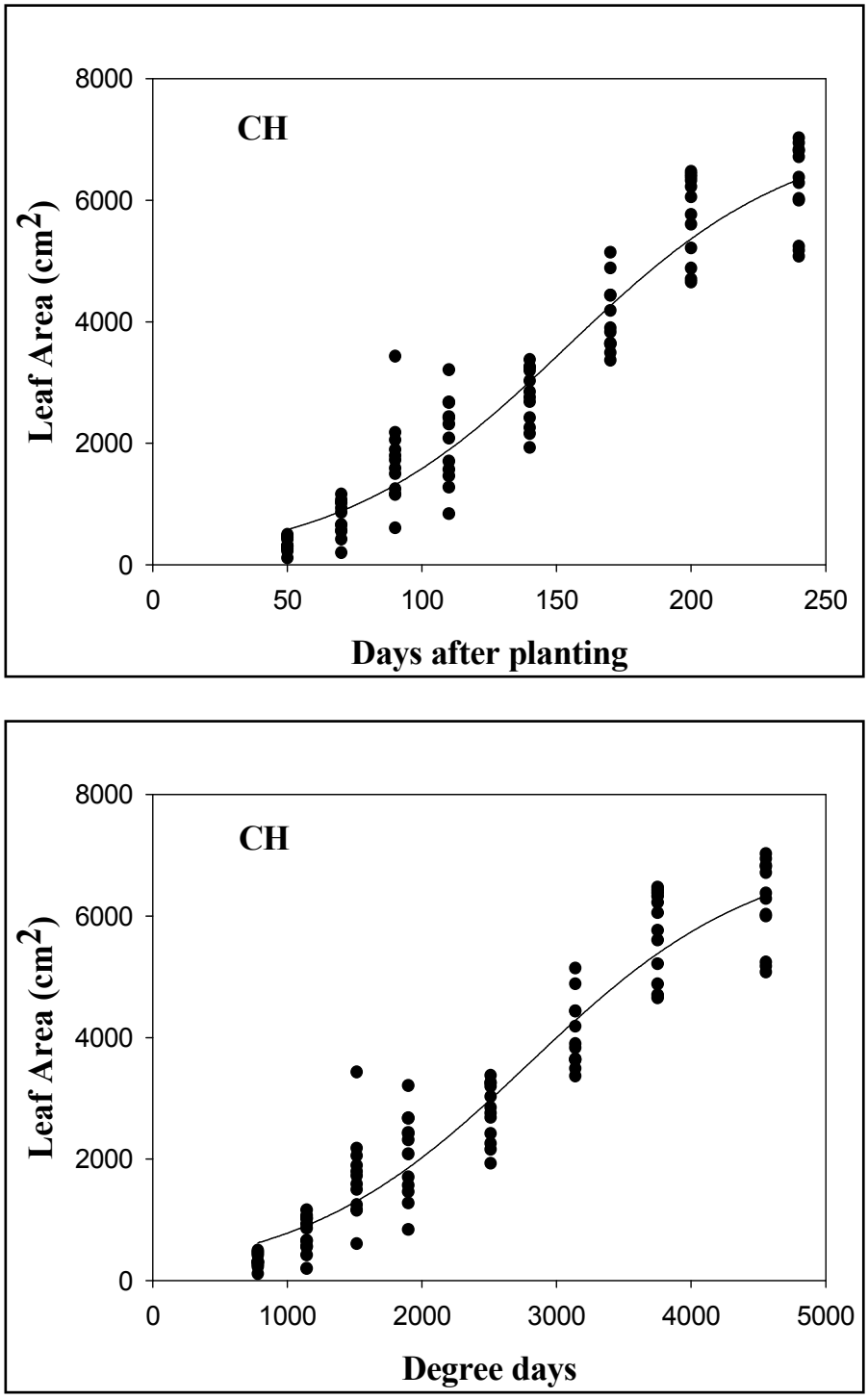
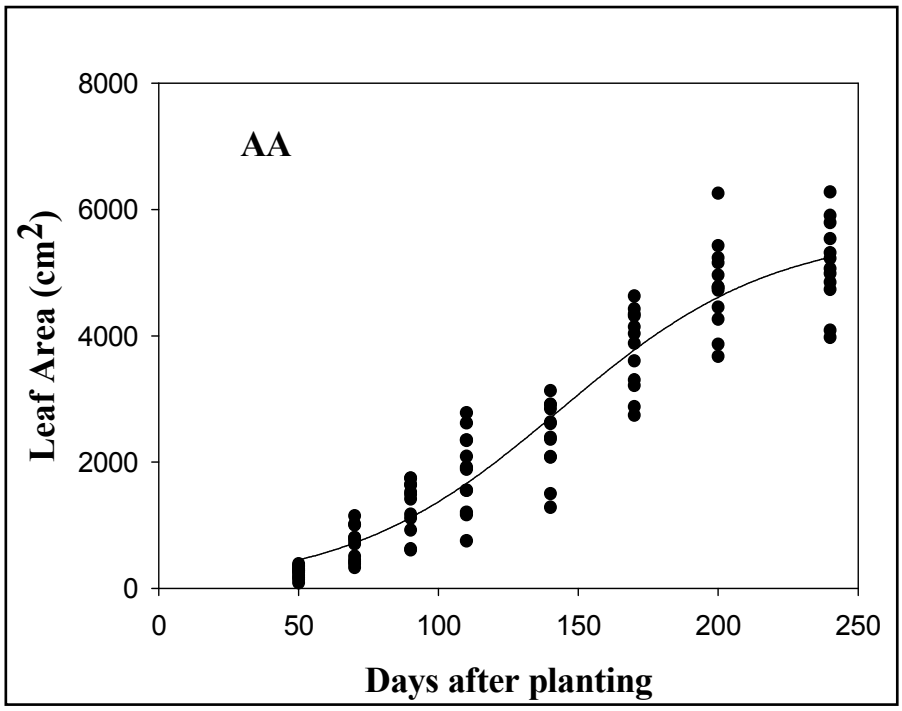


Figure 30. **Sigmoid model graphs of total leaf area for chamber treatment (CH) with days after planting (DAP) and degree days (DD).**



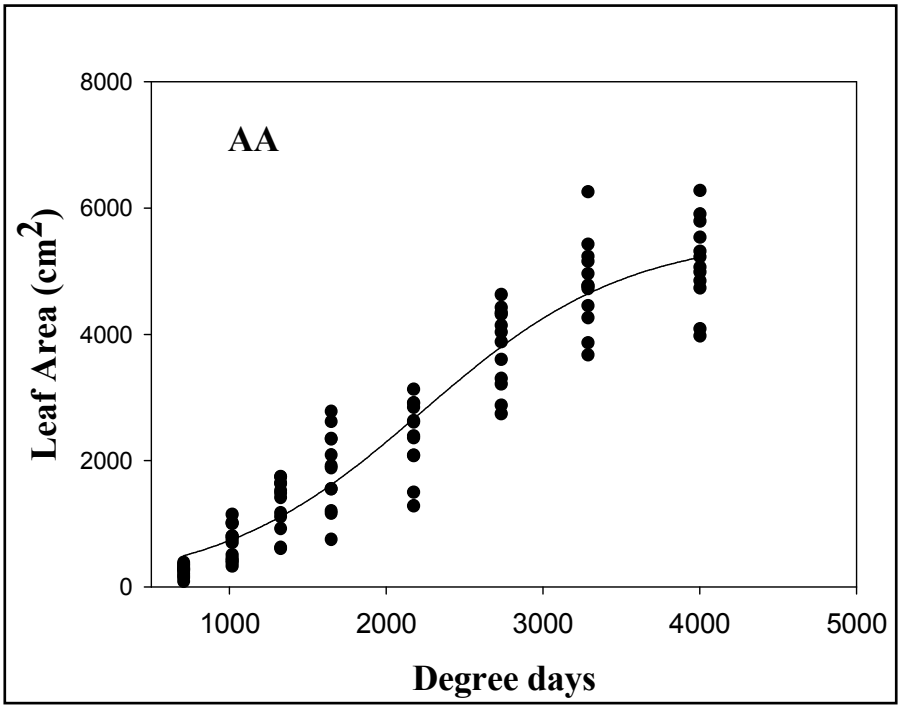


Figure 31. Sigmoid model graphs of total leaf area for ambient treatment (AA) with days after planting (DAP) and degree days (DD).

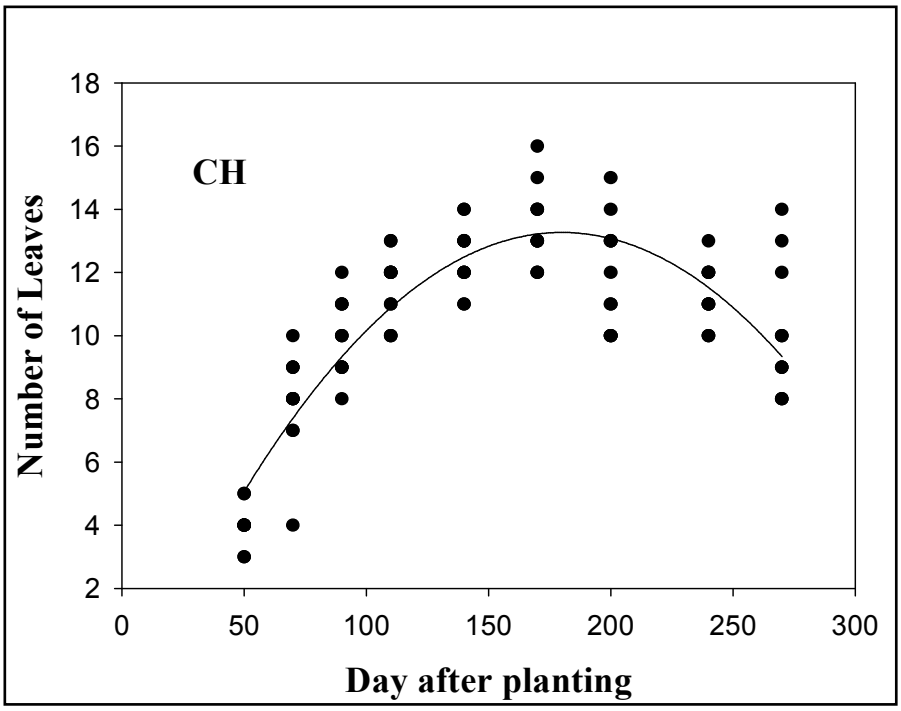
5.3.3 Number of Leaves

Number of leaves was best explained with the quadratic model. Table 7 shows the model equations for both treatments in terms of days after planting and degree days. The R^2 values were similar for both degree days and days after planting for both treatments. For the chamber treatment the R^2 values where 0.77 for DAP and 0.75 for DD while the ambient treatment had R^2 values of 0.69 and 0.66 for DAP and DD, respectively. The maximum production of leaves was reached at 170 DAP; from that day on leaves started to decline (Figure 32 and Figure 33). As the plant height increase so does the proportion of shaded leaves. Torres Justiniano (2005) and Guindin-Garcia (2003) observed a maximum of 15 leaves develop in sugarcane variety US-67-22-2. The sugarcane crop model CANEGRO studied by Guindín-García (2003) predicted only 11 leaves. Only a maximum of 13 leaves were observed in this thesis research at an early stage of the crop. Apparently, the senescence and decomposition rates surpass the leaf production rate due to the shorter distance between rows in the chambers and ambient plots.

Table 7. Predicting models for number of leaves of main stem

Model Equation	Treatment	Predictor	R^2	P-value
$L = -2.46 + 0.1748 * DAP - 0.0005 * DAP^2$	CH	DAP	0.77	<0.001
$L = -0.348 + 0.0081 * DD - 1.21e^{-6} * DD^2$	CH	DD	0.75	<0.001
$L = -1.98 + 0.1606 * DAP - 0.0005 * DAP^2$	AA	DAP	0.69	<0.001
$L = -0.104 + 0.0086 * DD - 1.49e^{-6} * DD^2$	AA	DD	0.66	<0.001

CH- chamber treatment, AA- ambient plot, DAP-days after planting, DD-degree days
L= number of leaves



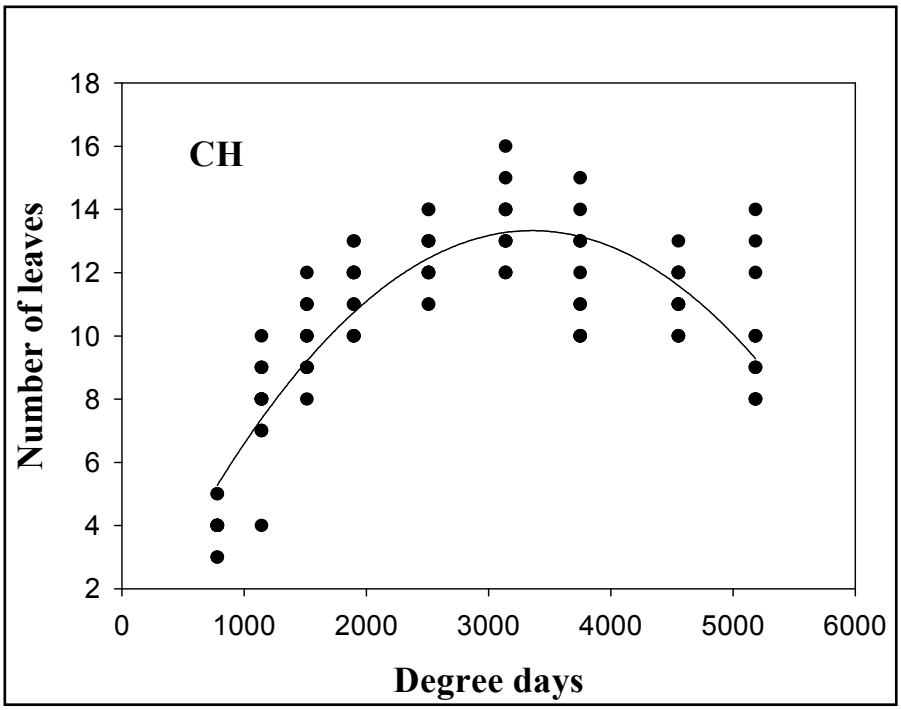


Figure 32. Quadratic model graphs of number of leaves for chamber treatment (CH) with days after planting (DAP) and degree days (DD).

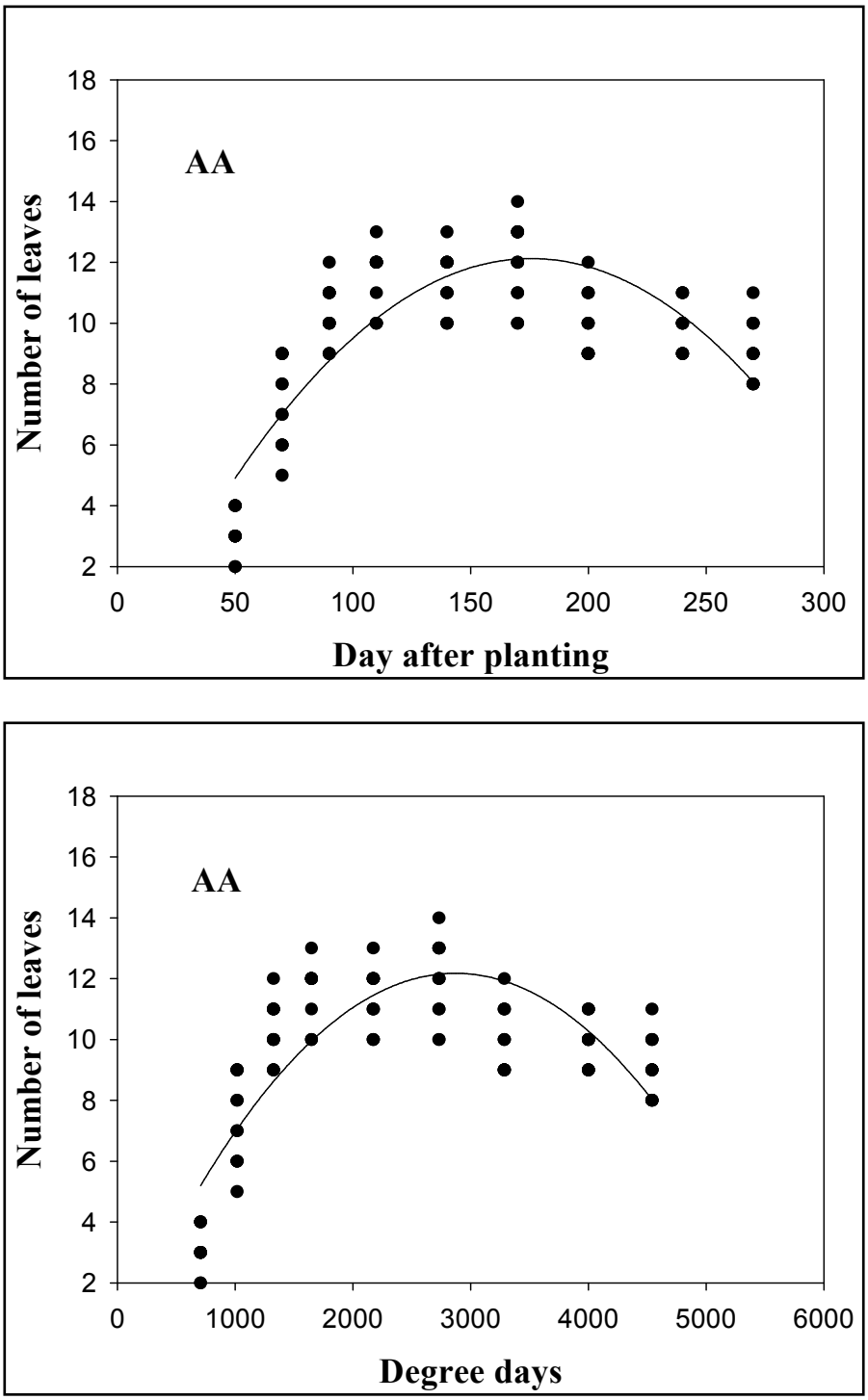


Figure 33. Quadratic model graphs of number of leaves for ambient treatment (AA) with days after planting (DAP) and degree days (DD).

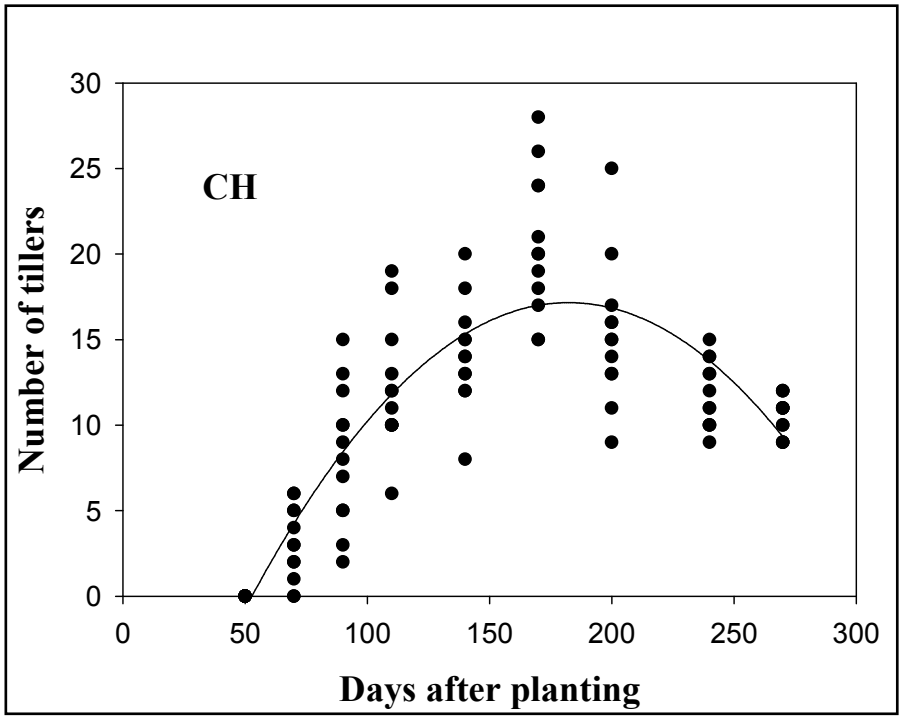
5.3.4 Number of Tillers

The number of tillers obtained the poorest fitting among all variables studied. The best fitting model for number of tillers, which showed consistency with the crop's physiological development on tillers, was the quadratic model. This model represents a rapid increase in the number of tillers at the beginning of the crop cycle reaching a maximum number of tillers at the maturation stage, followed by a decline apparently, due to light competition (Figure 34 and Figure 35). The maximum number of tillers was reached at 170 DAP or 3144 degree days for the chamber treatment and 2733 degree days for the ambient treatment. Torres Justiniano (2005) observed that 1500 degree days were needed to produce 4 tillers. In our investigation at 1550 degree days the chamber treatments had on average 4 tillers, which is similar to those found by Torres Justiniano (2005) at 1500 accumulated degree days although no decline was observed in the number of tillers in that study. Van Dillewijn (1952) identified two phases that can describe the behavior of our data. The first phase is characterized by an early sub-phase of profuse tillering and the second phases with a decline in tillering and more distinctive stalk elongation. Table 8 shows the model equations with its respective R^2 values and its statistical probability values.

Table 8. Predicting models for number of tillers

Model Equation	Treatment	Predictor	R^2	P-value
$T = -16.83 + 0.3727 * DAP - 0.0010 * DAP^2$	CH	DAP	0.74	<0.001
$T = -12.54 + 0.0176 * DD - 2.58e^{-6} * DD^2$	CH	DD	0.74	<0.001
$T = -15.86 + 0.3636 * DAP - 0.0010 * DAP^2$	AA	DAP	0.61	<0.001
$T = -12.04 + 0.0198 * DD - 3.477e^{-7} * DD^2$	AA	DD	0.60	<0.001

CH- chamber treatment, AA- ambient plot, DAP-days after planting, DD-degree days
T= number of tillers



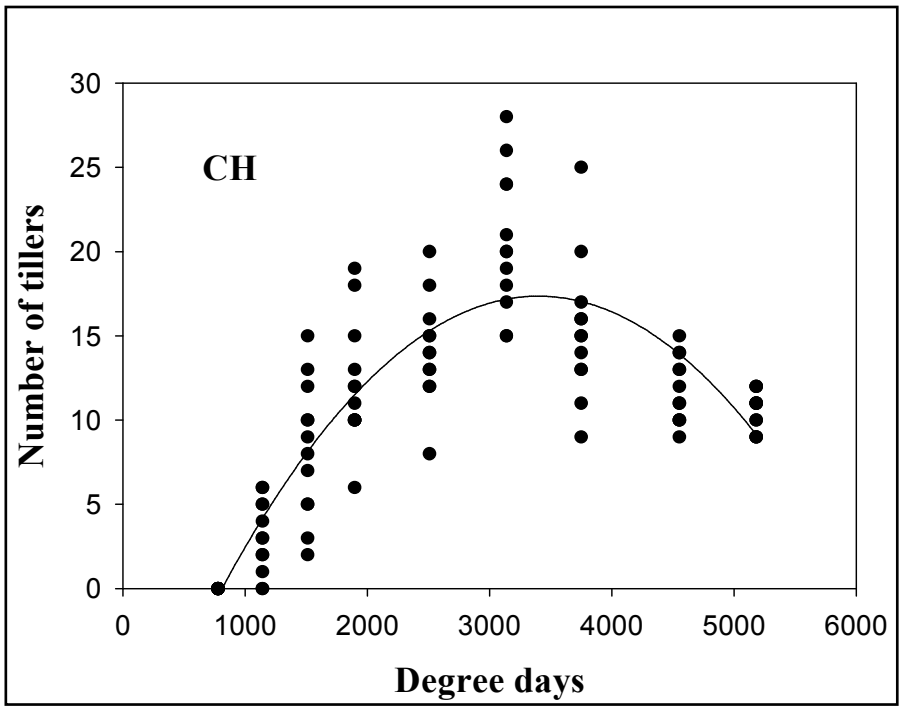
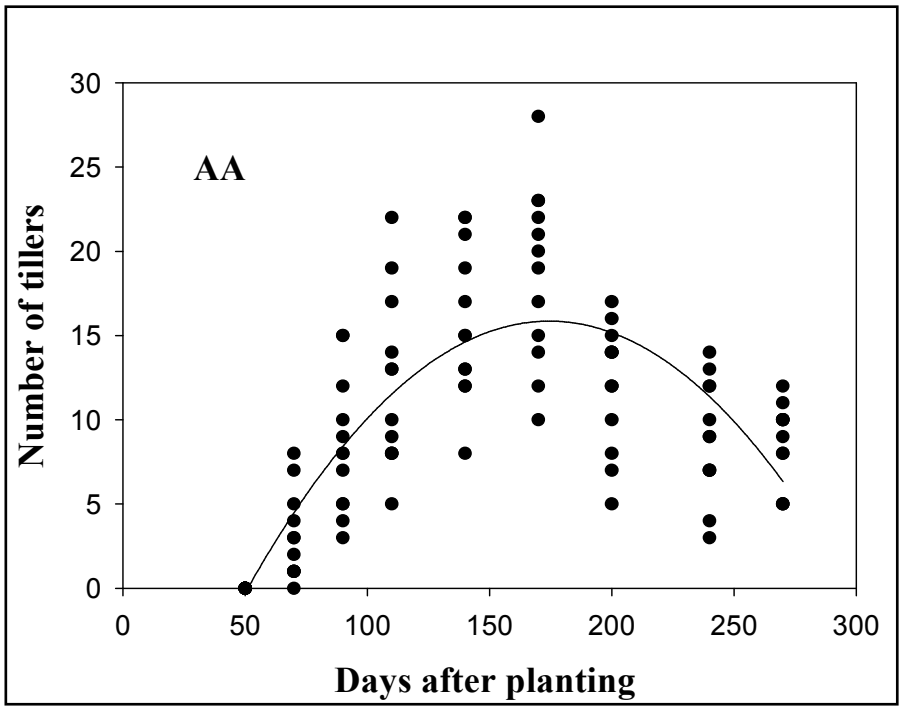


Figure 34. Quadratic model graphs of number of tillers for chamber treatment (CH) with days after planting (DAP) and degree days (DD).



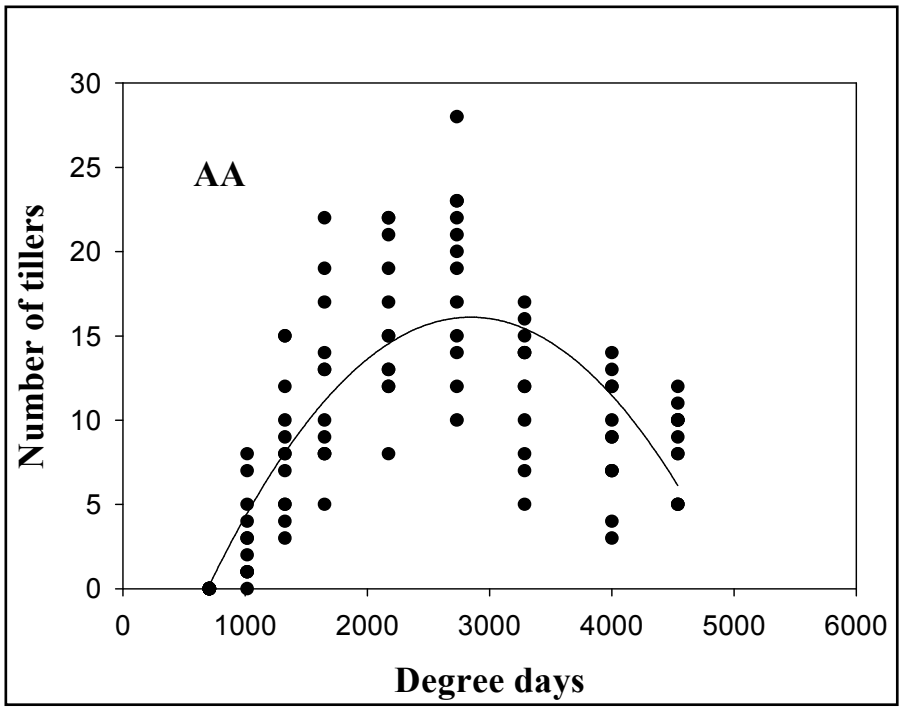


Figure 35. Quadratic model graphs of number of tillers for ambient treatment (AA) with days after planting (DAP) and degree days (DD).

6. SUMMARY AND CONCLUSIONS

This study was conducted to identify and measure differences between sugarcane US-67-22-2 grown in open-top chambers and ambient environments. Differences in mean air temperature were 1.3 ºC higher in the open-top chambers compare to the ambient plots. Night temperatures inside chambers were 0.9 º to 1 ºC higher than in the ambient plots. PAR inside the chambers were 18 to 20% less compared to the ambient plots as a result of absorption by the chambers plastic walls and the aluminum frame. This reductions level of PAR within chamber was not great enough to reduce leaf photosynthesis or leaf chlorophyll to the extent that growth between chambers and ambient plots were statistically significant. CO_2 concentrations were found to be very similar with ambient plot conditions.

The differences in the environment conditions between the open-top chambers and ambient plots did affect plant height but no effect on total above ground biomass was observed. Plant height was greater by 34% inside the chambers. This effect in higher plant height is consistent with other research investigations were chambers produced taller plants without difference in yield. Plant height increase inside the chambers may de due to the lack of sufficient mechanical stress due to wind gust or a faster accumulation of degree days inside the chambers. A reduction in the number of leaves and number of tillers was seen after 170 DAP. The variables of temperature and radiation have been used to explain the differences of biomass between open-top chambers and ambient plots in other research investigations. They stated that a reduction of PAR and the high night temperatures were the cause of the differences in biomass (Moya et al., 1997). The high night temperatures could increase the night-time respiration rates making the plant to loss the CO_2 capture during the day.

The best fitted model for height and leaf area in relation to days after planting and degree days, were the sigmoid-shaped model. The quadratic model best fitted and explained the development of number of leaves and number of tillers. Both days after planting and degree days showed similar correlation values. Most of the differences in these variables were seen after 170 DAP when the plant had passed the juvenile phase and had started its growth period.

Based on the literature review and this experiment it can be conclude that climatological differences impose on plants by chambers can result in either insignificant differences or great differences in growth or yield. The extent of the observed differences is probably related to the natural outside environment under which the plants are being grown as well the species studied and the chamber system used.

The open-top chambers does modified the environment but not enough to produce statistical significant measurements at plant aboveground biomass, total leaf area, tillering, number of leaves, photosynthesis and leaf chlorophyll content. It does affect significantly sugarcane plant height stimulating the plant to grow taller than sugarcane plants grown in ambient conditions. In spite of the climatological differences, open-top chambers provide a useful tool to study possible climate change impact on crops and offer the best available approach for investigating plant responses to CO_2 under field conditions.

This research has generated qualitative means to predict the effect of OTC in sugarcane research studies. Using these equations, researchers in the future will be able to account for the OTC effect and separate the truly impact of the research variables on sugarcane US-67-22-2.

7. RECOMMENDATIONS

1. Further research is needed to include parameters such as wind velocity, relative humidity, soil temperature, soil moisture and net radiation as this could contribute to the differences observed between the chambers and the ambient plots. A detail investigation of the changes in microclimate parameters would facilitate the development of a mathematical model which could be used in the extrapolation of results from chambers experiments to the open-field.

2. It is recommended to localize the sensors of photosynthetically active radiation, temperature and CO_2 at different height profiles especially at a height above canopy level.

3. More replications of open-top chambers and ambient plots are recommended to minimize the variability between data and maximize the precision of the measurements.

4. The use of OTCs is encourage to find better ways of plant adaptation to future changes in the environment.

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9. APPENDICES

Appendix A. Field Installation

Figure A1. Chamber fan

Figure A2. Field Site

Figure A3. Ambient plots

Figure A4. Sugarcane cuttings planting

Figure A5. Shed Installation

Figure A6. Open-top chambers installed

Appendix B. Instrumentation

Figure B1. Vacuum pump for CO_2 suction

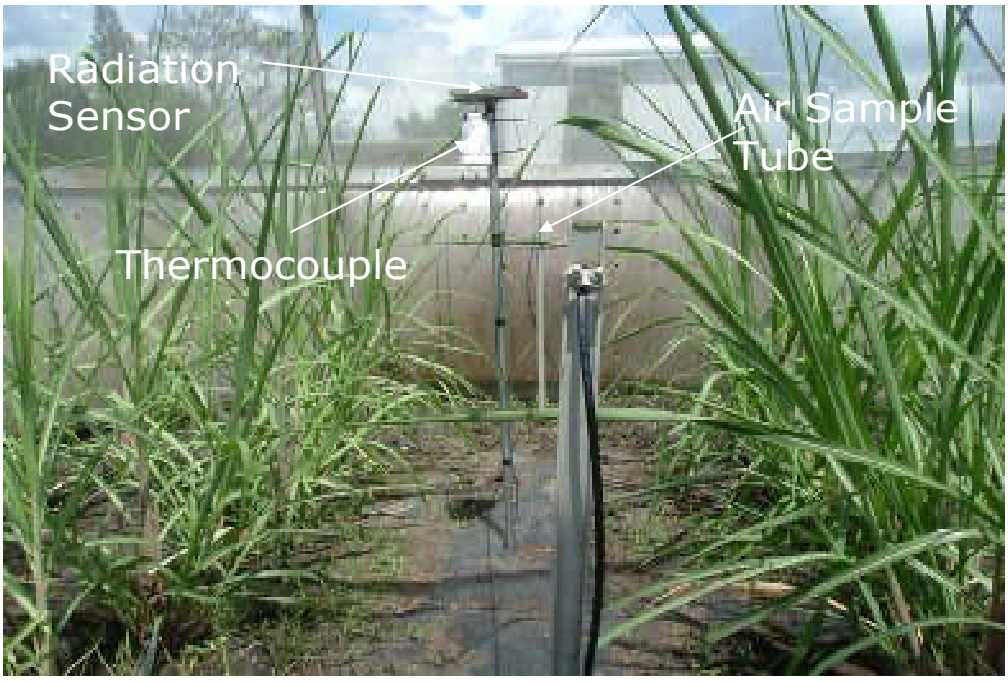

Figure B2. Solar radiation, temperature and CO_2 instruments

Figure B3. Instruments inside shed for monitoring chambers and ambient plots.

Appendix C. CR23X Program

```
;{ CR23X}
;
*Table 1 Program
   01: 15 Execution Interval (seconds)

1:  Batt Voltage (P10)
 1: 1        Loc [ Batt      ]

2:  If time is (P92)
 1: 0        Minutes (Seconds --) into a
 2: 1440 Interval (same units as above)
 3: 30      Then Do

    3:  Signature (P19)
     1: 2        Loc [ Prog_sig  ]

4:  End (P95)

5:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 1        F
 4: 30       Then Do

    6:  Set Port(s) (P20)
     1: 0000     C8, C7, C6, C5 Options
     2: 0010     C4..C1 = low/low/high/low

7:  End (P95)

8:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 2        F
 4: 30       Then Do

 Process for Chamber 1

; Measures LI-7000

    9:  Volt (Diff) (P2)
     1: 1        Reps
     2: 25       5000 mV, 60 Hz Reject, Fast Range (same as code 45)
```

```
 3: 3       DIFF Channel
 4: 7       Loc [A_CO2_1   ]
 5: 0.4     Mult
 6: 0.0     Offset

10:  Volt (Diff) (P2)
 1: 1       Reps
 2: 25      5000 mV, 60 Hz Reject, Fast Range (same as code 45)
 3: 4       DIFF Channel
 4: 8       Loc [A_H20_1   ]
 5: 0.01    Mult
 6: 0.0     Offset


11:  Volt (Diff) (P2)
 1: 1       Reps
 2: 22      50 mV, 60 Hz Reject, Slow Range
 3: 9       DIFF Channel
 4: 19      Loc [Qsensor1]
 5: 269.208 Mult
 6: 0.0     Offset

12:  Panel Temperature (P17)
 1: 4       Loc [ RefTemp   ]

13:  Thermocouple Temp (DIFF) (P14)
 1: 1       Reps
 2: 21      10 mV, 60 Hz Reject, Slow Range
 3: 5       DIFF Channel
 4: 1       Type T (Copper-Constantan)
 5: 4       Ref Temp (Deg. C) Loc [ RefTemp   ]
 6: 23      Loc [Thermo1   ]
 7: 1.8     Mult
 8: 32      Offset


14:  End (P95)


15:  If (X<=>F) (P89)
 1: 3       X Loc [Counter   ]
 2: 1       =
 3: 3       F
 4: 30      Then Do
```

```
16:  Set Port(s) (P20)
 1: 0000     C8, C7, C6, C5 Options
 2: 1000     C4..C1 = high/low/low/low


17:  End (P95)

18:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 4        F
 4: 30       Then Do

; Process for chamber 2

; Measures LI-7000

19:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       5000 mV, 60 Hz Reject, Fast Range (same as code 45)
 3: 3        DIFF Channel
 4: 9        Loc [A_CO2_2   ]
 5: 0.4      Mult
 6: 0.0      Offset

20:  Volt (Diff) (P2)
 1: 1        Reps
 2: 25       5000 mV, 60 Hz Reject, Fast Range (same as code 45)
 3: 4        DIFF Channel
 4: 10       Loc [A_H20_2   ]
 5: 0.01     Mult
 6: 0.0      Offset

21:  Volt (Diff) (P2)
 1: 1        Reps
 2: 22       50 mV, 60 Hz Reject, Slow Range
 3: 10       DIFF Channel
 4: 20       Loc [Qsensor2]
 5: 269.64   Mult
 6: 0.0      Offset

22:  Panel Temperature (P17)
 1: 4        Loc [RefTemp   ]

23:  Thermocouple Temp (DIFF) (P14)
```

```
    1: 1        Reps
    2: 21        10 mV, 60 Hz Reject, Slow Range
    3: 6        DIFF Channel
    4: 1        Type T (Copper-Constantan)
    5: 4        Ref Temp (Deg. C) Loc [ RefTemp   ]
    6: 24        Loc [Thermo2   ]
    7: 1.8       Mult
    8: 32        Offset

24:  End (P95)


   25:  If (X<=>F) (P89)
    1: 3        X Loc [Counter   ]
    2: 1        =
    3: 5        F
    4: 30        Then Do

       26:  Set Port(s) (P20)
        1: 0010     C8..C5 = low/low/high/low
        2: 0000     C4..C1 = low/low/low/low

   27:  End (P95)

   28:  If (X<=>F) (P89)
    1: 3        X Loc [Counter   ]
    2: 1        =
    3: 6        F
    4: 30        Then Do

; Process for chamber 3

; Measures LI-7000

       29:  Volt (Diff) (P2)
        1: 1        Reps
        2: 25        5000 mV, 60 Hz Reject, Fast Range (same as code 45)
        3: 3        DIFF Channel
        4: 11        Loc [A_CO2_3   ]
        5: 0.4       Mult
        6: 0.0       Offset

       30:  Volt (Diff) (P2)
        1: 1        Reps
        2: 25        5000 mV, 60 Hz Reject, Fast Range (same as code 45)
```

```
 3: 4        DIFF Channel
 4: 12       Loc [A_H20_3   ]
 5: 0.01     Mult
 6: 0.0      Offset

31:  Volt (Diff) (P2)
 1: 1        Reps
 2: 22       50 mV, 60 Hz Reject, Slow Range
 3: 11       DIFF Channel
 4: 21       Loc [Qsensor3]
 5: 282.53   Mult
 6: 0.0      Offset

32:  Panel Temperature (P17)
 1: 4        Loc [ RefTemp   ]

33:  Thermocouple Temp (DIFF) (P14)
 1: 1        Reps
 2: 21       10 mV, 60 Hz Reject, Slow Range
 3: 7        DIFF Channel
 4: 1        Type T (Copper-Constantan)
 5: 4        Ref Temp (Deg. C) Loc [ RefTemp   ]
 6: 25       Loc [Thermo3   ]
 7: 1.8      Mult
 8: 32       Offset

34:  End (P95)


35:  If (X<=>F) (P89)
 1: 3        X Loc [ Counter   ]
 2: 1        =
 3: 7        F
 4: 30       Then Do

36:  Set Port(s) (P20)
 1: 0000     C8..C5 = low/low/low/low
 2: 0001     C4..C1 = low/low/low/high

37:  End (P95)

38:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 8        F
```

```
 4: 30      Then Do

; Process for chamber 4

; Measures LI-7000

    39:  Volt (Diff) (P2)
     1: 1       Reps
     2: 25      5000 mV, 60 Hz Reject, Fast Range (same as code 45)
     3: 3       DIFF Channel
     4: 13      Loc [B_CO2_4   ]
     5: 0.4     Mult
     6: 0.0     Offset


    40:  Volt (Diff) (P2)
     1: 1       Reps
     2: 25      5000 mV, 60 Hz Reject, Fast Range (same as code 45)
     3: 4       DIFF Channel
     4: 14      Loc [B_H2O_4   ]
     5: 0.01    Mult
     6: 0.0     Offset


41:  End (P95)

42:  If (X<=>F) (P89)
 1: 3       X Loc [Counter   ]
 2: 1       =
 3: 9       F
 4: 30      Then Do

    43:  Set Port(s) (P20)
     1: 0000    C8..C5 = low/low/low/low
     2: 0100    C4..C1 = low/high/low/low

44:  End (P95)

45:  If (X<=>F) (P89)
 1: 3       X Loc [Counter   ]
 2: 1       =
 3: 10      F
 4: 30      Then Do
```

```
; Process for chamber 5

; Measures LI-7000

    46:  Volt (Diff) (P2)
     1: 1        Reps
     2: 25       5000 mV, 60 Hz Reject, Fast Range (same as code 45)
     3: 3        DIFF Channel
     4: 15       Loc [B_CO2_5   ]
     5: 0.4      Mult
     6: 0.0      Offset

    47:  Volt (Diff) (P2)
     1: 1        Reps
     2: 25       5000 mV, 60 Hz Reject, Fast Range (same as code 45)
     3: 4        DIFF Channel
     4: 16       Loc [B_H20_5   ]
     5: 0.01     Mult
     6: 0.0      Offset

    48:  Volt (Diff) (P2)
     1: 1        Reps
     2: 22       50 mV, 60 Hz Reject, Slow Range
     3: 12       DIFF Channel
     4: 22       Loc [Qsensor4]
     5: 268.77   Mult
     6: 0.0      Offset

    49:  Panel Temperature (P17)
     1: 4        Loc [ RefTemp   ]

    50:  Thermocouple Temp (DIFF) (P14)
     1: 1        Reps
     2: 21       10 mV, 60 Hz Reject, Slow Range
     3: 8        DIFF Channel
     4: 1        Type T (Copper-Constantan)
     5: 4        Ref Temp (Deg. C) Loc [ RefTemp   ]
     6: 26       Loc [Thermo4   ]
     7: 1.8      Mult
     8: 32       Offset

  51:  End (P95)
```

```
52:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 11        F
 4: 30       Then Do

    53:  Set Port(s) (P20)
     1: 0001     C8..C5 = low/low/low/high
     2: 0000     C4, C3, C2, C1 Options

54:  End (P95)

55:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 12        F
 4: 30       Then Do

; Process for chamber 6

; Measures LI-7000

    56:  Volt (Diff) (P2)
     1: 1        Reps
     2: 25        5000 mV, 60 Hz Reject, Fast Range (same as code 45)
     3: 3        DIFF Channel
     4: 17        Loc [B_CO2_6   ]
     5: 0.4      Mult
     6: 0.0      Offset

    57:  Volt (Diff) (P2)
     1: 1        Reps
     2: 25        5000 mV, 60 Hz Reject, Fast Range (same as code 45)
     3: 4        DIFF Channel
     4: 18        Loc [B_H20_6   ]
     5: 0.01      Mult
     6: 0.0      Offset

    58:  Panel Temperature (P17)
     1: 4        Loc [ RefTemp   ]

    59:  Thermocouple Temp (DIFF) (P14)
     1: 1        Reps
     2: 21        10 mV, 60 Hz Reject, Slow Range
     3: 1        DIFF Channel
```

```
    4: 1        Type T (Copper-Constantan)
    5: 4        Ref Temp (Deg. C) Loc [ RefTemp   ]
    6: 27       Loc [Thermo5   ]
    7: 1.8      Multiplier
    8: 32       Offset


60:  End (P95)

61:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 13       F
 4: 30       Then Do

    62:  Set Port(s) (P20)
     1: 0100     C8..C5 = low/high/low/low
     2: 0000     C4, C3, C2, C1 Options

63:  End (P95)

64:  If (X<=>F) (P89)
 1: 3        X Loc [Counter   ]
 2: 1        =
 3: 14       F
 4: 30       Then Do

    65:  Z=F x 10^n (P30)
     1: 0        F
     2: 00       n, Exponent of 10
     3: 3        Z Loc [Counter   ]

66:  End (P95)

67:  If time is (P92)
 1: 0        Minutes (Seconds --) into a
 2: 15       Interval (same units as above)
 3: 30       Then Do

    68:  Do (P86)
     1: 10       Set Output Flag High (Flag 0)

    69:  Set Active Storage Area (P80)
     1: 1        Final Storage Area 1
     2: 15       Arrays ID
```

```
70:  Real Time (P77)
 1: 1220     Year,Day,Hour/Minute (midnight = 2400)

71:  Sample (P70)
 1: 1        Reps
 2: 1        Loc [ Batt      ]

72:  Sample (P70)
 1: 1        Reps
 2: 7        Loc [A_CO2_1    ]

73:  Sample (P70)
 1: 1        Reps
 2: 8        Loc [A_H20_1    ]

74:  Sample (P70)
 1: 1        Reps
 2: 9        Loc [A_CO2_2    ]

75:  Sample (P70)
 1: 1        Reps
 2: 10       Loc [A_H20_2    ]

76:  Sample (P70)
 1: 1        Reps
 2: 11       Loc [A_CO2_3    ]

77:  Sample (P70)
 1: 1        Reps
 2: 12       Loc [A_H20_3    ]

78:  Sample (P70)
 1: 1        Reps
 2: 13       Loc [B_CO2_4    ]

79:  Sample (P70)
 1: 1        Reps
 2: 14       Loc [B_H2O_4    ]

80:  Sample (P70)
 1: 1        Reps
 2: 15       Loc [B_CO2_5    ]
```

```
81:  Sample (P70)
 1: 1        Reps
 2: 16       Loc [B_H20_5   ]

82:  Sample (P70)
 1: 1        Reps
 2: 17       Loc [B_CO2_6   ]

83:  Sample (P70)
 1: 1        Reps
 2: 18       Loc [B_H20_6   ]

84:  Sample (P70)
 1: 1        Reps
 2: 19       Loc [Qsensor1]

85:  Sample (P70)
 1: 1        Reps
 2: 20       Loc [Qsensor2]

86:  Sample (P70)
 1: 1        Reps
 2: 21       Loc [Qsensor3]

87:  Sample (P70)
 1: 1        Reps
 2: 22       Loc [Qsensor4]

88:  Sample (P70)
 1: 1        Reps
 2: 4        Loc [ RefTemp   ]

89:  Sample (P70)
 1: 1        Reps
 2: 23       Loc [Thermo1   ]

90:  Sample (P70)
 1: 1        Reps
 2: 24       Loc [Thermo2   ]

91:  Sample (P70)
 1: 1        Reps
 2: 25       Loc [Thermo3   ]
```

```
92:  Sample (P70)
 1: 1        Reps
 2: 26       Loc [Thermo4   ]

93:  Sample (P70)
 1: 1        Reps
 2: 27       Loc [Thermo5   ]

94:  End (P95)

95:  Z=Z+1 (P32)
 1: 3        Z Loc [Counter   ]
```

*Table 2 Program

02: 0 Execution Interval (seconds)

*Table 3 Subroutines

End Program

Appendix D. Data Sheet

Universidad de Puerto Rico
Recinto Universitario de Mayagüez

Nombre: _____
Fecha: _____
Hora: _____

# Chamber	# Fila	# Planta	Fotosíntesis	Clorofila	Area Foliar (m2)	# Total de Hojas	# Total de Hojas Verdes	# Total de Hojas Secas	Altura (cm)	# Brotes
1										
2										
3										
4										

5										
6										

Observaciones:

Chamber 1:

Chamber 2:

Chamber 3:

Chamber 4:

Chamber 5:

Chamber 6:

Appendix E. Tables of Physiological Measurements

Table E1. Plant Height

Height(cm)									
	DAP								
Treatment	50	70	90	110	140	170	200	240	270
CH	17 (±1.07)	28 (±1.76)	40 (±3.57)	55 (±5.17)	94 (±7.08)	166 (±11.24)	254 (±8.25)	327 (±11.43)	349 (±10.94)
AA	18 (±0.71)	24 (±1.65)	32 (±2.36)	43 (±3.08)	72 (±4.34)	141 (±5.70)	193 (±4.57)	233 (±5.70)	259 (±7.90)

* Values in parenthesis represent standard errors

Table E2. Number of Leaves

Number of leaves									
	DAP								
Treatment	50	70	90	110	140	170	200	240	270
CH	4 (±0.17)	8 (±0.43)	10 (±0.34)	12 (±0.31)	13 (±0.29)	13 (±0.36)	12 (±0.51)	11 (±0.28)	10 (±0.58)
AA	3 (±0.52)	7 (±2.08)	10 (±0.93)	12 (±1.00)	11 (±0.97)	12 (±1.64)	10 (±1.09)	10 (±0.63)	9 (±1.99)

*Values in parenthesis represent standard errors

Table E3. Number of Tillers

Number of Tillers									
	DAP								
Treatment	50	70	90	110	140	170	200	240	270
CH	0 (±0.00)	3 (±0.62)	8 (±1.16)	12 (±1.06)	14 (±0.89)	21(±1.21)	15 (±1.20)	12 (±0.56)	11 (±0.34)
AA	0 (±0.00)	3 (±0.74)	8 (±1.16)	12 (±1.48)	16 (±1.30)	19(±1.51)	12 (±1.09)	9 (±1.00)	9 (±0.70)

*Values in parenthesis represent standard errors

Table E4. Leaf Area

Leaf Area (cm^2)								
	DAP							
Treatment	50	70	90	110	140	170	200	240
CH	327	759	1694	2053	2763	4048	5723	6208
AA	257	692	1302	1850	2393	3791	4795	5144

Table E5. Leaf photosynthesis

Leaf Photosynthesis (µmol $m^{-2}s^{-1}$)						
	DAP					
Treatment	110	140	170	200	240	270
CH	34.33 (±1.70)	30.94 (±1.79)	29.46 (±1.83)	23.78 (±0.76)	23.88 (±1.43)	28.72 (±2.47)
AA	34.29 (±2.31)	34.5 (±1.41)	26.5 (±1.35)	21.06 (±0.91)	20.04 (±0.81)	22.03 (±1.44)

*Values in parenthesis represent standard errors

Table E6. Leaf chlorophyll content

Leaf Chlorophyll Content						
	DAP					
Treatment	110	140	170	200	240	270
CH	515	564	476	503	44	518
AA	610	578	598	568	456	526

Appendix F. Statistical Analysis for the Predicting Models

Table F1.1 Non-linear regression analysis for height DAP-CH

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9782	0.9568	0.9560	26.6949

	Coefficient	Std. Error	t	P	VIF
a	384.9757	13.1238	29.3342	<0.0001	6.5483<
b	37.6712	2.4410	15.4327	<0.0001	2.6984
x0	178.2353	3.9878	44.6946	<0.0001	5.6123<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	1659484.7381	829742.3690	1172.0887	<0.0001
Residual	105	74331.3638	707.9178		
Total	107	1733816.1019	16203.8888		

Table F1.2 Non-linear regression analysis for height DD-CH

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9783	0.9571	0.9563	26.6067

	Coefficient	Std. Error	t	P	VIF
a	384.4658	12.9104	29.7796	<0.0001	6.3969<
b	767.0233	48.7988	15.7181	<0.0001	2.6488
x0	3299.8694	80.3250	41.0815	<0.0001	5.5011<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	876879.5939	438439.7969	1695.7445	<0.0001
Residual	105	27148.0635	258.5530		
Total	107	904027.6574	8448.8566		

Table F1.3 Non-linear regression analysis for height DAP-AA

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9842	0.9686	0.9680	16.4315

	Coefficient	Std. Error	t	P	VIF
a	276.6372	7.2409	38.2050	<0.0001	5.7105<
b	37.6619	1.9938	18.8897	<0.0001	2.5079
x0	171.3481	3.1438	54.5028	<0.0001	4.8546<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	875678.3400	437839.1700	1621.6656	<0.0001
Residual	105	28349.3174	269.9935		
Total	107	904027.6574	8448.8566		

Table F1.4 Non-linear regression analysis for height DD-AA

Data Source: Data 1 in Notebook1
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9849	0.9700	0.9694	16.0796

	Coefficient	Std. Error	t	P	VIF
a	274.9320	6.8679	40.0315	<0.0001	5.4343<
b	670.1411	34.0861	19.6603	<0.0001	2.4623
x0	2747.4092	54.3593	50.5416	<0.0001	4.6790<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	876879.5939	438439.7969	1695.7445	<0.0001
Residual	105	27148.0635	258.5530		
Total	107	904027.6574	8448.8566		

Table F2.1 Non-linear regression analysis for Leaf area DAP-CH

Data Source: Data 1 in Notebook2
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9587	0.9191	0.9174	613.1248

	Coefficient	Std. Error	t	P	VIF
a	7188.7356	408.7628	17.5866	<0.0001	10.5878<
b	42.7073	3.7355	11.4329	<0.0001	3.3731
x0	154.0108	6.7410	22.8467	<0.0001	9.9120<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	397275180.4137	198637590.2069	528.4011	<0.0001
Residual	93	34960747.3918	375922.0150		
Total	95	432235927.8055	4549851.8716		

Table F2.2 Non-Linear regression analysis for Leaf area DD-CH

Data Source: Data 1 in Notebook3
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9583	0.9184	0.9166	615.9590

	Coefficient	Std. Error	t	P	VIF
a	7140.0284	400.0915	17.8460	<0.0001	10.1851<
b	855.9029	74.8166	11.4400	<0.0001	3.3605
x0	2795.0173	134.7313	20.7451	<0.0001	9.5583<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	396951221.1125	198475610.5563	523.1227	<0.0001
Residual	93	35284706.6930	379405.4483		
Total	95	432235927.8055	4549851.8716		

Table F2.3 Non-linear regression analysis for Leaf area DAP-AA

Data Source: Data 1 in Notebook4
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9533	0.9088	0.9069	555.5070

	Coefficient	Std. Error	t	P	VIF
a	5660.4510	273.3892	20.7047	<0.0001	6.8000<
b	38.1935	3.4706	11.0049	<0.0001	2.7223
x0	143.5476	5.5738	25.7540	<0.0001	5.9709<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	286105998.4775	143052999.2388	463.5727	<0.0001
Residual	93	28698690.7425	308588.0725		
Total	95	314804689.2200	3313733.5707		

Table F2.4 Non-linear regression analysis for Leaf area DD-AA

Data Source: Data 1 in Notebook5
Equation: Sigmoidal, Sigmoid, 3 Parameter
f= a/(1+exp(-(x-x0)/b))

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9533	0.9087	0.9068	555.8347

	Coefficient	Std. Error	t	P	VIF
a	5567.8533	251.2732	22.1586	<0.0001	5.9298<
b	654.1972	58.7569	11.1340	<0.0001	2.6099
x0	2232.4375	92.5628	24.1181	<0.0001	5.3076<

Analysis of Variance:

	DF	SS	MS	F	P
Regression	2	286072129.2086	143036064.6043	462.9714	<0.0001
Residual	93	28732560.0114	308952.2582		
Total	95	314804689.2200	3313733.5707		

Table F3.1 Quadratic regression analysis for number of leaves DAP-CH

Data Source: Data 1 in Notebook1
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8748	0.7653	0.7608	1.4578

	Coefficient	Std. Error	t	P	VIF
y0	-2.4639	0.7128	-3.4566	0.0008	25.8205<
a	0.1748	0.0103	16.9200	<0.0001	148.5372<
b	-0.0005	3.1898E-005	-15.2131	<0.0001	67.1824<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	727.5887	363.7943	171.1765	<0.0001
Residual	105	223.1521	2.1253		
Total	107	950.7407	8.8854		

Table F3.2 Quadratic regression analysis for number of leaves DD-CH

Data Source: Data 1 in Notebook1
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8666	0.7509	0.7462	1.5018

	Coefficient	Std. Error	t	P	VIF
y0	-0.3483	0.6184	-0.5632	0.5745	18.3099<
a	0.0081	0.0005	16.4339	<0.0001	112.0363<
b	-1.2133E-006	8.2327E-008	-14.7379	<0.0001	54.3969<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	713.9278	356.9639	158.2735	<0.0001
Residual	105	236.8129	2.2554		
Total	107	950.7407	8.8854		

Table F3.3 Quadratic regression analysis for number of leaves DAP-AA

Data Source: Data 1 in Notebook1
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8331	0.6940	0.6882	1.5440

	Coefficient	Std. Error	t	P	VIF
y0	-1.9824	0.7550	-2.6259	0.0099	25.8205<
a	0.1606	0.0109	14.6820	<0.0001	148.5372<
b	-0.0005	3.3784E-005	-13.5283	<0.0001	67.1824<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	567.7601	283.8801	119.0801	<0.0001
Residual	105	250.3139	2.3839		
Total	107	818.0741	7.6456		

Table F3.4 Quadratic regression analysis for number of leaves DD-AA

Data Source: Data 1 in Notebook1
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8111	0.6580	0.6514	1.6324

	Coefficient	Std. Error	t	P	VIF
y0	-0.1041	0.6877	-0.1514	0.8799	19.1690<
a	0.0086	0.0006	13.6316	<0.0001	116.4775<
b	-1.4902E-006	1.1850E-007	-12.5756	<0.0001	56.1614<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	538.2624	269.1312	100.9921	<0.0001
Residual	105	279.8117	2.6649		
Total	107	818.0741	7.6456		

Table F3.4 Quadratic regression analysis for number of tillers DAP-CH

Data Source: Data 1 in Number of tillers quadratic
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8614	0.7419	0.7370	3.3908

	Coefficient	Std. Error	t	P	VIF
y0	-16.8340	1.6580	-10.1535	<0.0001	25.8205<
a	0.3727	0.0240	15.5158	<0.0001	148.5372<
b	-0.0010	7.4193E-005	-13.7718	<0.0001	67.1824<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	3470.7618	1735.3809	150.9354	<0.0001
Residual	105	1207.2382	11.4975		
Total	107	4678.0000	43.7196		

Table F3.4 Quadratic regression analysis for number of tillers DD-CH

Data Source: Data 1 in Number of tillers quadratic
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.8612	0.7416	0.7367	3.3931

	Coefficient	Std. Error	t	P	VIF
y0	-12.5454	1.3971	-8.9796	<0.0001	18.3099<
a	0.0176	0.0011	15.7000	<0.0001	112.0361<
b	-2.5858E-006	1.8601E-007	-13.9013	<0.0001	54.3968<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	3469.1173	1734.5586	150.6587	<0.0001
Residual	105	1208.8827	11.5132		
Total	107	4678.0000	43.7196		

Table F3.4 Quadratic regression analysis for number of tillers DAP-AA

Data Source: Data 1 in Number of tillers quadratic
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7819	0.6114	0.6039	4.1557

	Coefficient	Std. Error	t	P	VIF
y0	-15.8567	2.0319	-7.8037	<0.0001	25.8205<
a	0.3636	0.0294	12.3488	<0.0001	148.5372<
b	-0.0010	9.0928E-005	-11.4607	<0.0001	67.1824<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	2852.3621	1426.1810	82.5835	<0.0001
Residual	105	1813.3046	17.2696		
Total	107	4665.6667	43.6044		

Table F3.4 Quadratic regression analysis for number of tillers DD-AA

Data Source: Data 1 in Number of tillers quadratic
Equation: Polynomial, Quadratic
f=y0+a*x+b*x^2

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7772	0.6040	0.5964	4.1950

	Coefficient	Std. Error	t	P	VIF
y0	-12.0499	1.7673	-6.8182	<0.0001	19.1689<
a	0.0198	0.0016	12.2675	<0.0001	116.4772<
b	-3.4770E-006	3.0452E-007	-11.4181	<0.0001	56.1611<

Analysis of Variance:

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	2	2817.9121	1408.9560	80.0650	<0.0001
Residual	105	1847.7546	17.5977		
Total	107	4665.6667	43.6044		

Appendix G. Statistical Analysis for the Physiological Measurements

Table G1. Analysis of temperature for sunny and cloudy day

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Temperature	40	0.99	0.98	0.98

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	110.04	21	5.24	77.82	<0.0001	
tipo	78.65	1	78.65	96.97	<0.0001	(tipo>dia)
tipo>dia	14.60	18	0.81	12.04	<0.0001	
trt	13.12	1	13.12	194.87	<0.0001	
tipo*trt	3.67	1	3.67	54.45	<0.0001	
Error	1.21	18	0.07			
Total	111.25	39				

Test:LSD Fisher Alfa:=0.05 DMS:=0.59832

Error: 0.8111 gl: 18

tipo	Medias	n		
NUB	25.17	20	A	
SOL	27.97	20		B

Letras distintas indican diferencias significativas(p<= 0.05)

Test:LSD Fisher Alfa:=0.05 DMS:=0.24381

Error: 0.0673 gl: 18

tipo	trt	Medias	n				
NUB	AA	24.90	10	A			
NUB	ch	25.44	10		B		
SOL	AA	27.10	10			C	
SOL	ch	28.85	10				D

Letras distintas indican diferencias significativas(p<= 0.05)

Table G2. Analysis of Plant Height

Análisis de la varianza

Variable	N	R²	R² Aj	CV
RAIZ_Height(cm)	216	0.99	0.98	6.14

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor (Error)
Modelo	5338.42	39	136.88	344.68	<0.0001
trt	85.10	1	85.10	14.92	0.0181(trt>rep)
trt>rep	22.82	4	5.70	1.49	0.2458(trt*rep>pl)
trt*rep>pl	68.74	18	3.82	9.62	<0.0001
DAP	5114.87	8	639.36	1609.94	<0.0001
trt*DAP	46.89	8	5.86	14.76	<0.0001
Error	69.90	176	0.40		
Total	5408.31	215			

Contrastes

trt*DAP	SC	gl	CM	F	p-valor (Error)
Contraste1	0.03	1	0.03	0.08	0.7728
Contraste2	0.95	1	0.95	2.39	0.1243
Contraste3	2.52	1	2.52	6.35	0.0126
Contraste4	4.08	1	4.08	10.28	0.0016
Contraste5	8.41	1	8.41	21.18	<0.0001
Contraste6	5.40	1	5.40	13.61	0.0003
Contraste7	24.17	1	24.17	60.87	<0.0001
Contraste8	47.08	1	47.08	118.55	<0.0001
Contraste9	39.33	1	39.33	99.05	<0.0001
Total	131.99	9	14.67	36.93	<0.0001

Table G3. Analysis of Number of leaves

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Number of leaves	216	0.88	0.85	11.29

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor (Error)
Modelo	1591.15	39	40.80	33.11	<0.0001
trt	39.19	1	39.19	8.91	0.0405(trt>rep)
trt>rep	17.59	4	4.40	1.70	0.1937(trt*rep>pl)
trt*rep>pl	46.56	18	2.59	2.10	0.0076
DAP	1464.58	8	183.07	148.58	<0.0001
trt*DAP	23.23	8	2.90	2.36	0.0197
Error	216.85	176	1.23		
Total	1808.00	215			

Contrastes

trt*DAP	SC	gl	CM	F	p-valor	(Error)
Contraste1	4.17	1	4.17	3.38	0.0676	
Contraste2	1.50	1	1.50	1.22	0.2714	
Contraste3	0.67	1	0.67	0.54	0.4630	
Contraste4	0.00	1	0.00	0.00	>0.9999	
Contraste5	8.17	1	8.17	6.63	0.0109	
Contraste6	12.04	1	12.04	9.77	0.0021	
Contraste7	20.17	1	20.17	16.37	0.0001	
Contraste8	10.67	1	10.67	8.66	0.0037	
Contraste9	5.04	1	5.04	4.09	0.0446	
Total	62.42	9	6.94	5.63	<0.0001	

Table G4. Analysis of Number of Tillers

Análisis de la varianza

Variable	N	R^2	R^2 Aj	CV
Number of Tillers	216	0.88	0.85	25.15

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	8235.06	39	211.16	32.13	<0.0001	
trt	48.17	1	48.17	3.02	0.1572	(trt>rep)
trt>rep	63.78	4	15.94	0.26	0.9014	(trt*rep>pl)
trt*rep>pl	1115.44	18	61.97	9.43	<0.0001	
DAP	6876.83	8	859.60	130.79	<0.0001	
trt*DAP	130.83	8	16.35	2.49	0.0139	
Error	1156.78	176	6.57			
Total	9391.83	215				

Contrastes

trt*DAP	SC	gl	CM	F	p-valor	(Error)
Contraste1	0.00	1	0.00	0.00	>0.9999	
Contraste2	0.04	1	0.04	0.01	0.9366	
Contraste3	0.17	1	0.17	0.03	0.8737	
Contraste4	0.00	1	0.00	0.00	>0.9999	
Contraste5	15.04	1	15.04	2.29	0.1321	
Contraste6	22.04	1	22.04	3.35	0.0688	
Contraste7	66.67	1	66.67	10.14	0.0017	
Contraste8	51.04	1	51.04	7.77	0.0059	
Contraste9	24.00	1	24.00	3.65	0.0576	
Total	179.00	9	19.89	3.03	0.0022	

Table G5. Analysis of Leaf Area

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Leaf Area(cm2)	192	0.96	0.95	16.94

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	722354542.06	37	19523095.73	90.78	<0.0001	
trt	8432221.72	1	8432221.72	6.19	0.0676	(trt>rep)
trt>rep	5447067.76	4	1361766.94	1.50	.2377	(trt*rep>pl)
trt*rep>pl	16096824.50	18	894268.03	4.16	<0.0001	
DAP	686394493.91	7	98056356.27	455.96	<0.0001	
trt*DAP	5983934.17	7	854847.74	3.98	0.0005	
Error	33118448.01	154	215054.86			
Total	755472990.07	191				

Contrastes

trt*DAP	SC	gl	CM	F	p-valor	(Error)
Contraste1	29237.48	1	29237.48	0.14	0.7128	
Contraste2	27302.53	1	27302.53	0.13	0.7221	
Contraste3	922007.05	1	922007.05	4.29	0.0401	
Contraste4	249624.32	1	249624.32	1.16	0.2830	
Contraste5	819770.96	1	819770.96	3.81	0.0527	
Contraste6	396862.95	1	396862.95	1.85	0.1763	
Contraste7	5174326.36	1	5174326.36	24.06	<0.0001	
Contraste8	6797024.25	1	6797024.25	31.61	<0.0001	
Total	14416155.89	8	1802019.49	8.38	<0.0001	

Table G6. Analysis of Photosynthesis

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Photosynthesis	144	0.58	0.45	20.01

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	4515.96	33	136.85	4.53	<0.0001	
trt	161.18	1	161.18	4.29	0.1071	(trt>rep)
trt>rep	150.33	4	37.58	1.13	0.3735	(trt*rep>pl)
trt*rep>pl	598.51	18	33.25	1.10	0.3606	
DAP	3237.32	5	647.46	21.45	<0.0001	
trt*DAP	368.61	5	73.72	2.44	0.0386	
Error	3320.53	110	30.19			
Total	7836.49	143				

Contrastes

trt*DAP	SC	gl	CM	F	p-valor(Error)
Contraste1	0.01	1	0.01	3.5E-04	0.9852
Contraste2	75.97	1	75.97	2.52	0.1155
Contraste3	52.51	1	52.51	1.74	0.1899
Contraste4	44.55	1	44.55	1.48	0.2270
Contraste5	88.74	1	88.74	2.94	0.0892
Contraste6	268.00	1	268.00	8.88	0.0036
Total	529.79	6	88.30	2.93	0.0110

Table G7. Analysis of Chlorophyll Content

Variable	N	R^2	R^2 Aj	CV
Chlorophyll	144	0.34	0.14	22.41

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	797910.19	33	24179.10	1.72	0.0199	
trt	100172.25	1	100172.25	4.76	0.0945	(trt>rep)
trt>rep	84164.31	4	21041.08	1.18	0.3531	(trt*rep>pl)
trt*rep>pl	321090.00	18	17838.33	1.27	0.2241	
DAP	221828.31	5	44365.66	3.15	0.0107	
trt*DAP	70655.33	5	14131.07	1.00	0.4193	
Error	1549299.36	110	14084.54			
Total	2347209.56	143				

Contrastes

trt*DAP	SC	gl	CM	F	p-valor	(Error)
Contraste1	53392.67	1	53392.67	3.79	0.0541	
Contraste2	1107.04	1	1107.04	0.08	0.7797	
Contraste3	89304.00	1	89304.00	6.34	0.0132	
Contraste4	25610.67	1	25610.67	1.82	0.1803	
Contraste5	988.17	1	988.17	0.07	0.7916	
Contraste6	425.04	1	425.04	0.03	0.8624	
Total	170827.58	6	28471.26	2.02	0.0687	

Table G8. Sugarcane Harvest

Análisis de la varianza

Variable	N	R²	R² Aj	CV
Stem Fresh Weight (kg/m2)	12	0.69	0.15	34.82

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	555.55	7	79.36	1.27	0.4312	
trt	0.14	1	0.14	1.4E-03	0.9722	(trt>rep)
trt>rep	410.73	4	102.68	1.64	0.3208	
cosecha	139.81	1	139.81	2.24	0.2089	
trt*cosecha	4.86	1	4.86	0.08	0.7940	
Error	249.70	4	62.43			
Total	805.25	11				

Variable	N	R²	R² Aj	CV
Stem dry weight (kg/m2)	12	0.68	0.12	42.18

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	39.37	7	5.62	1.22	0.4479	
trt	8.48	1	8.48	1.71	0.2613	(trt>rep)
trt>rep	19.86	4	4.97	1.08	0.4717	
cosecha	9.96	1	9.96	2.16	0.2154	
trt*cosecha	1.06	1	1.06	0.23	0.6561	
Error	18.42	4	4.61			
Total	57.79	11				

Variable	N	R²	R² Aj	CV
Leaf Apex Fresh Weight(kg/m2)	12	0.69	0.14	32.80

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	13.09	7	1.87	1.25	0.4374	
trt	3.13	1	3.13	1.46	0.2938	(trt>rep)
trt>rep	8.59	4	2.15	1.44	0.3666	
cosecha	0.77	1	0.77	0.51	0.5137	
trt*cosecha	0.60	1	0.60	0.40	0.5597	
Error	5.97	4	1.49			
Total	19.07	11				

Variable	N	R²	R² Aj	CV
Leaf Apex dry weight(kg/m2)	12	0.75	0.32	30.77

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	1.26	7	0.18	1.75	0.3083	
trt	0.63	1	0.63	6.69	0.0609	(trt>rep)
trt>rep	0.37	4	0.09	0.90	0.5377	
cosecha	0.13	1	0.13	1.24	0.3280	
trt*cosecha	0.14	1	0.14	1.32	0.3146	
Error	0.41	4	0.10			
Total	1.68	11				

Variable	N	R²	R² Aj	CV
Green leaves fresh weight(kg/m2)	12	0.76	0.34	34.29

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	5.20	7	0.74	1.80	0.2972	
trt	0.15	1	0.15	0.21	0.6676	(trt>rep)
trt>rep	2.71	4	0.68	1.65	0.3205	
cosecha	2.29	1	2.29	5.55	0.0779	
trt*cosecha	0.05	1	0.05	0.12	0.7434	
Error	1.65	4	0.41			
Total	6.84	11				

Variable	N	R²	R² Aj	CV
Green leaves dry weight(kg/m2)	12	0.81	0.49	29.44

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	0.75	7	0.11	2.49	0.1972	
trt	0.02	1	0.02	0.19	0.6850	(trt>rep)
trt>rep	0.40	4	0.10	2.36	0.2128	
cosecha	0.32	1	0.32	7.50	0.0519	
trt*cosecha	2.7E-03	1	2.7E-03	0.06	0.8138	
Error	0.17	4	0.04			
Total	0.92	11				

Variable	N	R²	R² Aj	CV
Dry leaves fresh weight(kg/m2)	12	0.55	0.00	44.64

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	1.38	7	0.20	0.71	0.6758	
trt	0.26	1	0.26	0.98	0.3782	(trt>rep)
trt>rep	1.04	4	0.26	0.94	0.5243	
cosecha	0.04	1	0.04	0.15	0.7172	
trt*cosecha	0.04	1	0.04	0.15	0.7172	
Error	1.11	4	0.28			
Total	2.49	11				

Variable	N	R²	R² Aj	CV
Dry leaves dry weight (kg/m2)	12	0.53	0.00	47.32

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	0.95	7	0.14	0.66	0.7071	
trt	0.14	1	0.14	0.76	0.4314	(trt>rep)
trt>rep	0.74	4	0.18	0.89	0.5448	
cosecha	0.07	1	0.07	0.34	0.5914	
trt*cosecha	4.0E-03	1	4.0E-03	0.02	0.8959	
Error	0.83	4	0.21			
Total	1.78	11				

Variable	N	R²	R² Aj	CV
Total fresh weight(kg/m2)	12	0.68	0.13	32.07

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	769.75	7	109.96	1.23	0.4445	
trt	9.24	1	9.24	0.06	0.8197	(trt>rep)
trt>rep	624.06	4	156.01	1.75	0.3011	
cosecha	129.96	1	129.96	1.46	0.2942	
trt*cosecha	6.50	1	6.50	0.07	0.8007	
Error	357.20	4	89.30			
Total	1126.95	11				

Variable	N	R²	R² Aj	CV
Total dry weight(kg/m2)	12	0.68	0.13	35.79

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor	(Error)
Modelo	67.22	7	9.60	1.23	0.4441	
trt	17.81	1	17.81	1.92	0.2379	(trt>rep)
trt>rep	37.07	4	9.27	1.19	0.4352	
cosecha	10.38	1	10.38	1.33	0.3127	
trt*cosecha	1.95	1	1.95	0.25	0.6430	
Error	31.16	4	7.79			
Total	98.38	11				