

## Research Article

# Leaf Growth and Canopy Development of Three Sugarcane Genotypes under High Temperature Rainfed Conditions in Northeastern Mexico

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The aim of this study was to compare sugarcane (*Saccharum* spp.) canopy developmental components of three commercial varieties (CP 72-2086, Mex 79-431, and Mex 68-P-23) in a subtropical environment under rainfed and high temperature conditions, a poorly described topic in the literature. A field experiment was carried out in southern Tamaulipas, Mexico, throughout November 2011–January 2013 crop cycle, during which 111 of the days had daily maximum temperatures at or above 35°C. Number of leaves, leaf area, leaf appearance rate, and leaf area index (LAI) were determined. Thermal time exposure, °Cd (°C day<sup>-1</sup>), was determined based on total number of green ligulate leaves using 10°C as the base temperature. At 5000°Cd the number of leaves per plant ranged from 32 to 40 and the dependence of leaf emergence rate as a function of temperature was confirmed. The leaf emergence rate of CP 72-2086 was significantly greater than that of the other two varieties. Cultivars did not differ with respect to leaf length but differed for all other parameters measured. These results show the potential importance of considering sugarcane varietal differences in leaf phenology and canopy development for breeding programs focusing on rainfed and high temperature conditions.

## 1. Introduction

Sugarcane (*Saccharum* spp.) production is an important component of the economy in many countries in the tropics and subtropics [1]. In Tamaulipas, Mexico, productivity has historically tended to be low under rainfed conditions but that has been exacerbated in recent years due to climate change which has resulted in less predictable rainfall distribution patterns. This, coupled with a significant number of very high maximum daily temperatures in the region has resulted in the need for further research leading to the development of varieties adapted to both rainfed conditions and high temperatures. In Mexico, sugarcane cultivation covers 777,242 hectares [2] and supplies raw material to 57 sugar mills in 15 states throughout the country [3]. Rainfed agriculture represents 59% of the total area in which sugarcane is grown in Mexico.

Plant development and growth are basic processes that define crop yield. Development refers to events during crop ontogeny including cell differentiation, organ initiation (organogenesis) and appearance (morphogenesis), and crop senescence. Growth refers to increases in organ or whole-plant physical dimensions such as length, height, area, and volume [4]. Plant development and growth are different but related processes [5]. Leaf appearance rate, leaf number, development stage throughout the growing season, and duration of developmental phase are examples of development parameters of interest in many agronomic studies [6]. Leaf development is characterized by the appearance of new leaves and the consequent increase in accumulated leaf number on a stem or on a whole-plant basis, whereas leaf growth is often measured as the increase in leaf area. Growth rate is proportional to the amount of solar radiation intercepted.

Therefore, leaf area development and growth are critical in determining the canopy leaf area index (LAI) to maximize the interception of solar radiation by the crop canopy for photosynthesis, leading to the accumulation of biomass [7], which finally defines crop yield [8]. Hence, slow or inefficient development of the plant canopy may be critical in limiting the final yield produced by a sugarcane crop [9].

Not only development but also maintenance of leaf area is necessary component for efficient radiation interception and transformation into chemical energy during photosynthesis and consequently for attaining maximum potential yield of crops [10]. As the major factor driving leaf area expansion is temperature (assuming sufficient soil water is available), then leaf area is derived from the relationship between temperature and leaf appearance rate and the relationship between leaf number and leaf area [11]. Thus, the development of the sugarcane canopy is dependent on the rates of tillering, leaf appearance, and leaf expansion [12].

Therefore estimation of leaf area is most critical during the period of early growth when the canopy is not yet closed and only a portion of incident radiation is intercepted. However, it is also important to know the rate of leaf appearance throughout the entire crop cycle to accurately apportion dry matter to emerging leaves [5]. Sugarcane can produce more than 40 fully expanded leaves on a single stem in a 10–14-month cropping cycle [11]. Leaf appearance in sugarcane has additional significance because with each leaf comes an internode, the commercially important storage component in a sugarcane crop [5].

An important function of crop physiological research is to quantify the role of various growth processes and climatic elements contributing to yield variation. To quantify key physiological parameters for sugarcane, it is necessary to establish growth analysis parameters by regularly sampling the crop throughout the growing season. However, there are few data on yield accumulation in plant or ratoon-crops of sugarcane crops growing under rainfed and high temperature conditions [13].

Understanding how plants respond to their environment requires knowledge of the morphological and anatomical changes that take place during development and the molecular and biochemical mechanisms underlying those changes. A critical and practical requirement for conducting such analyses is to develop an accurate description of the developmental stages of the entire plant and its organs (phenology) so that plant sampling for analyses is highly repeatable. A major element needed for such an understanding is a detailed description of the changes in size, composition, and organization of the plant canopy during plant development [14]. Most field studies to date in subtropical regions in which leaf and canopy development have been described were conducted under maximum temperatures not considered to be extreme. For example, in a study conducted in Brazil [8], the maximum temperatures reported during the growing season ranged from 30 to 35°C. Another study [12], conducted in Sri Lanka, reported maximum average temperatures of 32°C. Similar studies on sugarcane leaf and canopy development in other subtropical areas of the world have reported maximum temperatures of 29°C in the USA [15] and 24°C in Australia [11].

One study in Australia under glasshouse conditions reported temperatures up to 38°C [16] but there was no indication of how many days of 38°C were experienced by the sugarcane plants. In the sugarcane production areas of southern Tamaulipas, Mexico, maximum temperatures can be much higher. Based on weather data records collected by our weather station (see Section 2 herein), during 2010 there were 109 days above 35°C and 6 days above 40°C. During 2011 there were 168 days above 35°C and 11 days above 40°C with an absolute maximum of 45°C. These data suggested that further research is needed to address how rainfed conditions combined with high temperatures affect canopy development in sugarcane. The aim of this study was to compare leaf and canopy development components in three commercial varieties of sugarcane in a subtropical environment under rainfed and high temperature conditions.

## 2. Materials and Methods

A field experiment was carried out in Northeastern Mexico in the southern region of the state of Tamaulipas within the municipality of Ocampo (22°51'N, 99°20'W, and 325 m altitude) during November 2011–January 2013 cropping season. This location has a (A)Ca(m)(w)(e)gw'' (subtropical extreme semiwarm) climate, according to Köppen System [17]. Typically, the commercial sugarcane crop in this area begins with planting in early fall (September) and vegetative growth takes place during the following months until the end of spring (June). Harvest typically occurs during the months of November and December.

Three commercial varieties were studied in this experiment: Mex 68-P-23, CP72-2086, and Mex 79-431. The varieties were selected for their wide range of phenotypic growth characteristics and because they are all widely grown in the southern region of Tamaulipas. Based on fertilizer applications for the region as recommended by the local sugar mill, the crop was fertilized during planting in November of 2012 at a rate of 60-60-60 (NPK) kg ha<sup>-1</sup> using a commercially available 10-17-19 mix. The crop was fertilized again in March two months after plant emergence at a rate of 60-0-0 using a commercially available 23-00-00 mix. Weeds were mechanically controlled as needed throughout the experiment in order to minimize biotic stress due to weed competition. Diseases and insects were not controlled because pest populations were below economic thresholds.

Vegetative seed-cane pieces of each variety were planted in rows on November 29, 2011. Emergence was determined in each plot by counting the number of emerged plants, on a daily basis, in all plots. The crop emergence date was considered as the day when about 50% of the plants had emerged from the soil with a maximum plant density of 10–12 plants m<sup>-1</sup>. Three weeks after crop emergence, 10 plants in the two central rows of each plot were randomly selected and tagged with a unique number for identification throughout the experiment. White paint marks on leaves were used to identify new leaves on each plant progressively up the stem every 15 days. Marked plants were used to determine leaf appearance rate, leaf number, and leaf area. Leaf appearance

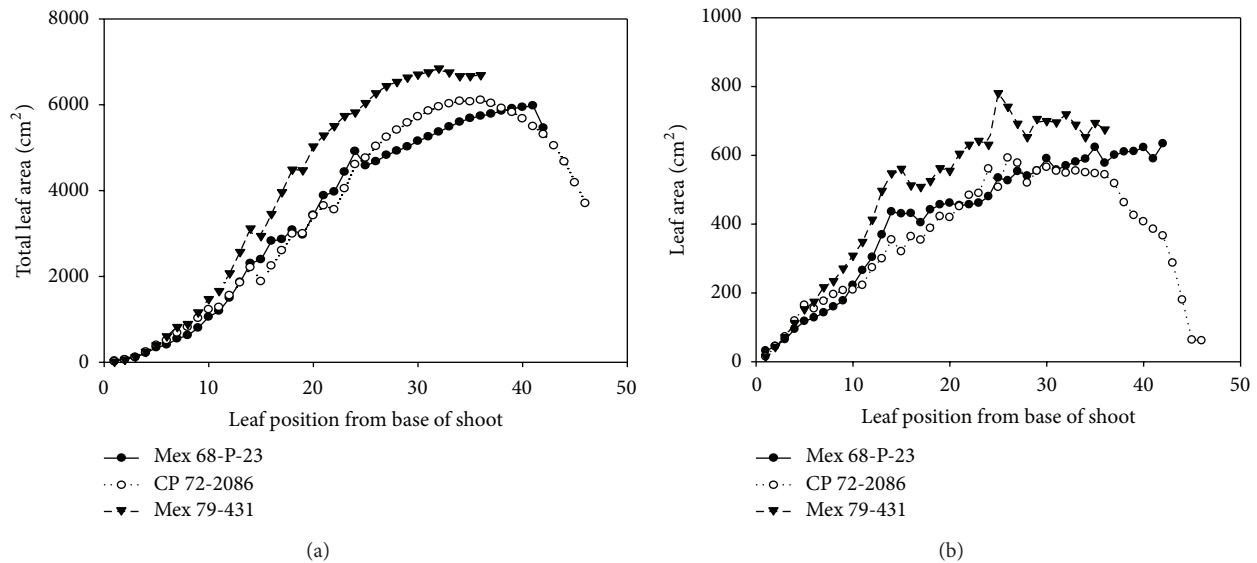


FIGURE 1: Total leaf area (a) and individual leaf area (b) in the field experiment for the three sugarcane cultivars tested under high temperature rainfed conditions in Northeastern Mexico.

rate was estimated by counting weekly the number of visible green expanded leaves per plant (from January 2012 until January 2013). Leaf appearance rate was estimated by the time elapsed between the emergence of each leaf. Full leaf expansion was assumed when the ligule was visible [14]. Blade length from ligule to blade tip and maximum blade width of individual expanded leaves were measured nondestructively after ligule appearance in all marked plants, and green leaf area ( $\text{cm}^2$ ) of these expanded leaves was calculated as follows: leaf area = (length  $\times$  width)  $\times$  0.71 as suggested by van Oosterom et al. [18] and Singels et al. [15]. Leaf area index (LAI) was determined for each plot with the LAI-2000 Plant Canopy Analyzer (LI-COR, Lincoln, NE) according to the instructions described in the LAI-2000 user manual [19]. All readings were taken with the instrument level and at 1.5 m above the ground early in the morning or late in the afternoon under overcast sky conditions. Briefly, the measurement protocol used a sensor with a  $45^\circ$  view cap with two diagonal transects per plot and each measurement consists of an average of 10 individual readings. The two diagonal transects were averaged to produce a single reading per plot.

Rainfall amount, maximum temperature ( $T_{\max}$ ), and minimum temperature ( $T_{\min}$ ) were recorded during the experiment using a Hobo Weather Station (Onset Computer Corp., Bourne, MA) data logger once per hour and placed at 50 m of the experiment. Thermal time (TT,  $^\circ\text{Cd}$ ) was calculated as the accumulation of daily mean temperature  $((T_{\max} + T_{\min})/2)$  minus the base temperature assumed as  $10^\circ\text{C}$  [20]. Accumulated thermal time was determined from the date of emergence of the primary shoot until development of the flag leaf.

The experimental layout was a randomized complete block design with three replicates. Each replicate consisted of four 10 m long rows planted in a north-south direction.

Results where appropriate were subjected to one-way analysis of variance (ANOVA) using locally developed statistical software (Emilio Olivares Saenz, Universidad Autónoma de Nuevo Leon). If the ANOVA showed that there were significant differences at  $P < 0.05$ , then means were compared using Tukey's studentized range test (HSD) and indicated on the figure with different letters for each mean.

### 3. Results and Discussion

Sugarcane leaves are the main path for water loss via transpiration, absorption of photosynthetically active radiation (PAR), and C assimilation via photosynthesis. These processes together allow for the provision of energy and organic molecules necessary for growth and development of the whole plant. Leaf area determines the amount of incident PAR intercepted by the crop canopy and ultimately dry matter production [7], and the transformation of solar energy into chemical energy during photosynthesis is directly related to yield [10].

Results showed that there were significant differences ( $P < 0.05$ ) between cultivars in terms of the total leaf area, leaf width, area per leaf, leaf number, and LAI. The total leaf area of green leaves (ligulate and actively photosynthetic) increased for all cultivars in a sigmoidal pattern (Figure 1(a)) in agreement with the data of Robertson and coworkers [11] and Sinclair and coworkers [20]. Total leaf area per plant began to diverge between varieties early in development and the differences became significant by the developmental stage represented by node #10. The variety which achieved the greatest total leaf area was Mex 79-431, with a total of  $6900 \text{ cm}^2$  by the time node #32 being developed. The other two varieties achieved a lower total leaf area with CP 72-2086 reaching a maximum at  $6200 \text{ cm}^2$  by node #35 and Mex 68-P-23 maximizing at  $6000 \text{ cm}^2$  by node #41. These results showed

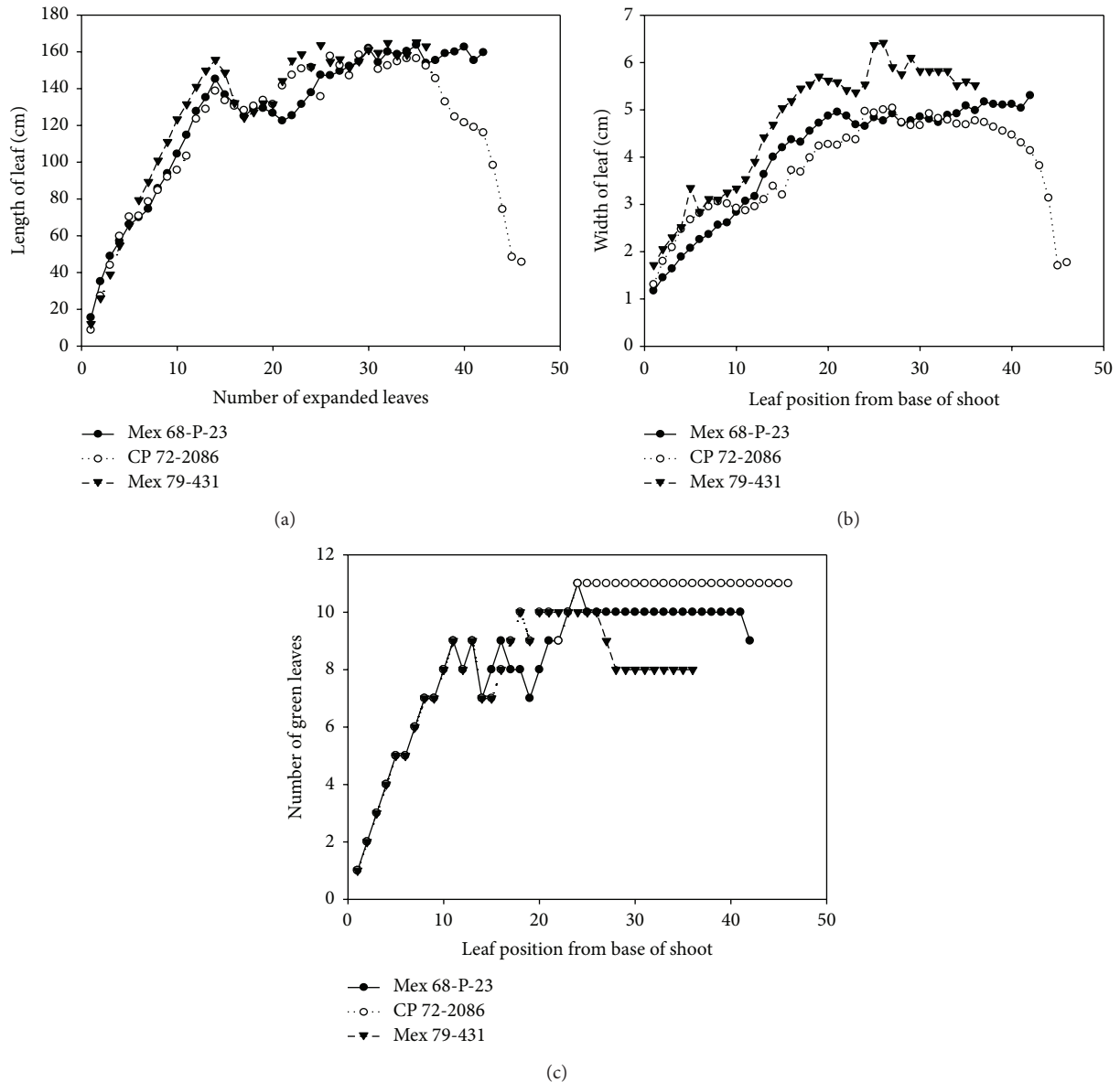


FIGURE 2: Length (a), width (b) of each leaf lamina, and total number of leaves (c) for the three sugarcane cultivars under high temperature rainfed conditions in Northeastern Mexico.

that the variety of sugarcane significantly affected not only the total leaf area per plant but also the timing of maximum leaf area development. All three varieties showed a tendency to decrease in total leaf area after reaching a maximum.

The maximum area of individual leaves depends largely on the order in which they appear along the stalk with the younger leaves producing the greatest area and to a lesser extent on the availability of PAR [9, 11]. The maximum area of individual leaves (Figure 1(b)) was reached at a much lower node in CP 72-2086 than in Mex 79-431 and Mex 68-P-23. Variety CP 72-2086 reached a maximum area of individual leaves at approximately leaf #26, while Mex 68-P-23 did not reach maximum leaf area until about leaf #35. After leaf #15 in all varieties, a 30-day period of drought affected the total leaf area and maximum area of individual leaves, visible as a dip

in Figure 1(a) and more clearly in Figure 1(b), in agreement with previous reports [21–23].

Significant differences in the total number of leaves per plant were found showing that Mex 79-431 had 36 leaves, Mex 68-P-23 had 42 leaves, and CP 72-2086 had 46 leaves by the end of the experiment (Table 1). All cultivars were found to have the same ( $P > 0.05$ ) length of leaves (Figure 2(a)). However, Mex 79-431 had wider leaves ( $P < 0.05$ ) than CP 72-2086 and Mex 68-P-23 (Figure 2(b)), and this compensated to some extent for the smaller number of green leaves of this cultivar.

The maximum width for an individual leaf was reached in Mex 79-431 at leaf #26, significantly greater than the leaf widths of the other two varieties tested. Mex 68-P-23 and CP 72-2086 had the same maximum leaf width by leaf #20.



TABLE 1: Days from emergence to harvest, total number of leaves, leaf appearance rate, thermal time, and accumulated thermal time ( $\pm$ standard error) for three sugarcane cultivars in Northeastern Mexico under high temperature rainfed conditions.

Cultivar	Emergence to harvest (days)	Total number of leaves	Leaf appearance rate (leaves·day <sup>-1</sup> )	Thermal time (°C·day <sup>-1</sup> ·leaf <sup>-1</sup> )	Accumulated thermal time (°Cd)
Mex 68-P-23	378.7 $\pm$ 0.98 <sup>a</sup>	41.7 $\pm$ 0.27 <sup>b</sup>	0.11 $\pm$ 0.0003 <sup>b</sup>	135.4 $\pm$ 0.90 <sup>b</sup>	5562.1 $\pm$ 4.53 <sup>a</sup>
CP 72-2086	356.3 $\pm$ 1.19 <sup>b</sup>	46.0 $\pm$ 0.41 <sup>a</sup>	0.13 $\pm$ 0.0004 <sup>a</sup>	120.8 $\pm$ 1.06 <sup>c</sup>	5557.2 $\pm$ 4.77 <sup>a</sup>
Mex 79-431	365.0 $\pm$ 1.25 <sup>b</sup>	35.6 $\pm$ 0.27 <sup>c</sup>	0.10 $\pm$ 0.0003 <sup>c</sup>	154.4 $\pm$ 1.20 <sup>a</sup>	5557.2 $\pm$ 5.50 <sup>a</sup>
Mean	366.7	41.3	0.113	136.9	5558.8
CV (%)	2.13	2.15	2.25	2.38	2.31

\*Means  $\pm$  SE within a parameter not followed by the same letter are significantly different at  $P \leq 0.05$  according to Tukey's multiple range test.

The number of green leaves on plants of all three varieties increased until each had approximately 10 green leaves. After this point, the number of green leaves remained more or less constant as each new leaf appeared due to an equivalent loss of an older leaf through the process of senescence.

This pattern (maintaining approximately 10 green leaves) was maintained in Mex 68-P-23 and CP 72-2086 until about leaf #20 and then increased to 11 green leaves throughout the rest of the life of the plant (Figure 2(c)). However, for variety Mex 79-431 the number of green leaves increased to 10 but was reduced to 8 leaves after about leaf #26 and remained constant at 8 leaves throughout the rest of the life of the plant. In terms of development, maximum leaf length was reached at leaf 30–35 for all three varieties, whereas maximum leaf area and leaf width were reached at leaves 26–27. Maximum leaf area was about 634, 593, and 781 cm<sup>2</sup> for cultivars Mex 68-P-23, CP 72-2086, and Mex 79-431, respectively, at leaf #42, leaf #26, and leaf #25, respectively. From leaf #12 through #36, cultivar Mex 79-431 developed the greatest leaf area and produced the widest leaves (leaves #14 through #24) (Figure 2(b)).

Maximum LAI occurred at about 328 days after emergence in the cultivars CP 72-2086 (3.6) and Mex 79-431 (3.0) and at about 300 days after emergence in the cultivar Mex 68-P-23 (3.23) (Figure 3). The maximum LAI was generally attained when the stalk had 36–38 fully expanded leaves with maxima varying widely (Figure 3), demonstrating that the development of the sugarcane canopy is dependent on leaf appearance, leaf expansion, and leaf size [12]. Our results showed that radiation interception of the cultivars Mex 68-P-23 and Mex 79-431 could be increased significantly by adjusting planting density to optimally match the cultivar and crop start date as suggested by Singels and coworkers [15]. A production system where sugarcane is harvested annually as occurs in Tamaulipas could possibly benefit from this practice.

The role of temperature in determining leaf appearance rate in sugarcane has been well studied [5, 8, 9, 11, 15, 16, 20, 24]. Thermal time has been found to correlate with the appearance of sugarcane leaves [9]. Consequently, a faster leaf appearance rate under a high temperature regime may seem intuitive [16].

Thermal time was derived by accumulating the differences between  $T_{\text{mean}}$  and base temperature ( $T_b = 10^\circ\text{C}$ ) each day from the date of emergence. Figure 4 shows strong effects of temperature on final leaf size as a function of leaf position.

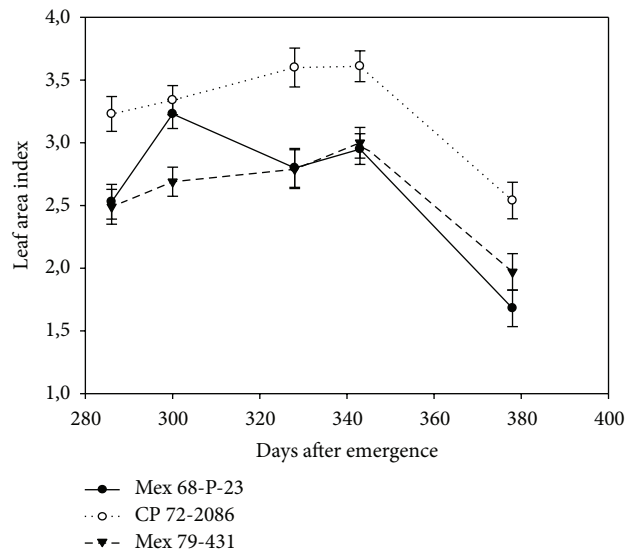


FIGURE 3: The progress of leaf area index with time for three sugarcane cultivars sown on the same date during the period canopy closure to harvest under high temperature rainfed conditions in Northeastern Mexico.

In agreement with Bonnett and coworkers [16], under higher temperatures all cultivars produced more leaves and, in this experiment, the number of fully expanded leaves increased nonlinearly with cumulative thermal time (Figure 4). The mean of thermal requirement from sowing to appearance of the first leaf was significantly different ( $P < 0.05$ ) for the three cultivars (Figure 4(a)): 474.6, 449.4, and 488.1 °Cd for Mex 68-P-23, CP 72-2086, and Mex 79-431, respectively. Leaf appearance of the cultivars occurred during the period between 379, 356, and 365 days after emergence for Mex 68-P-23, CP 72-2086, and Mex 79-431, respectively (Table 1). This period coincided with commercial harvest in agreement with sugar mill and flowering stage in CP 72-2086 (Figures 4(b) and 4(c)). From emergence to harvest, the thermal time increased from 75 °Cd at leaf #1 to 174 °Cd at leaf #16, to 148 °Cd at leaf #30, and to 90 °Cd at leaf #40 (Figure 4(b)). Hence the mean of the total thermal (°Cd) requirement from planting to emergence of leaf #1 was 470 °Cd and this period coincided with cool weather.

Leaf appearance rate declined as a function of cumulative thermal time, so that, at emergence, leaves took 75 °Cd to

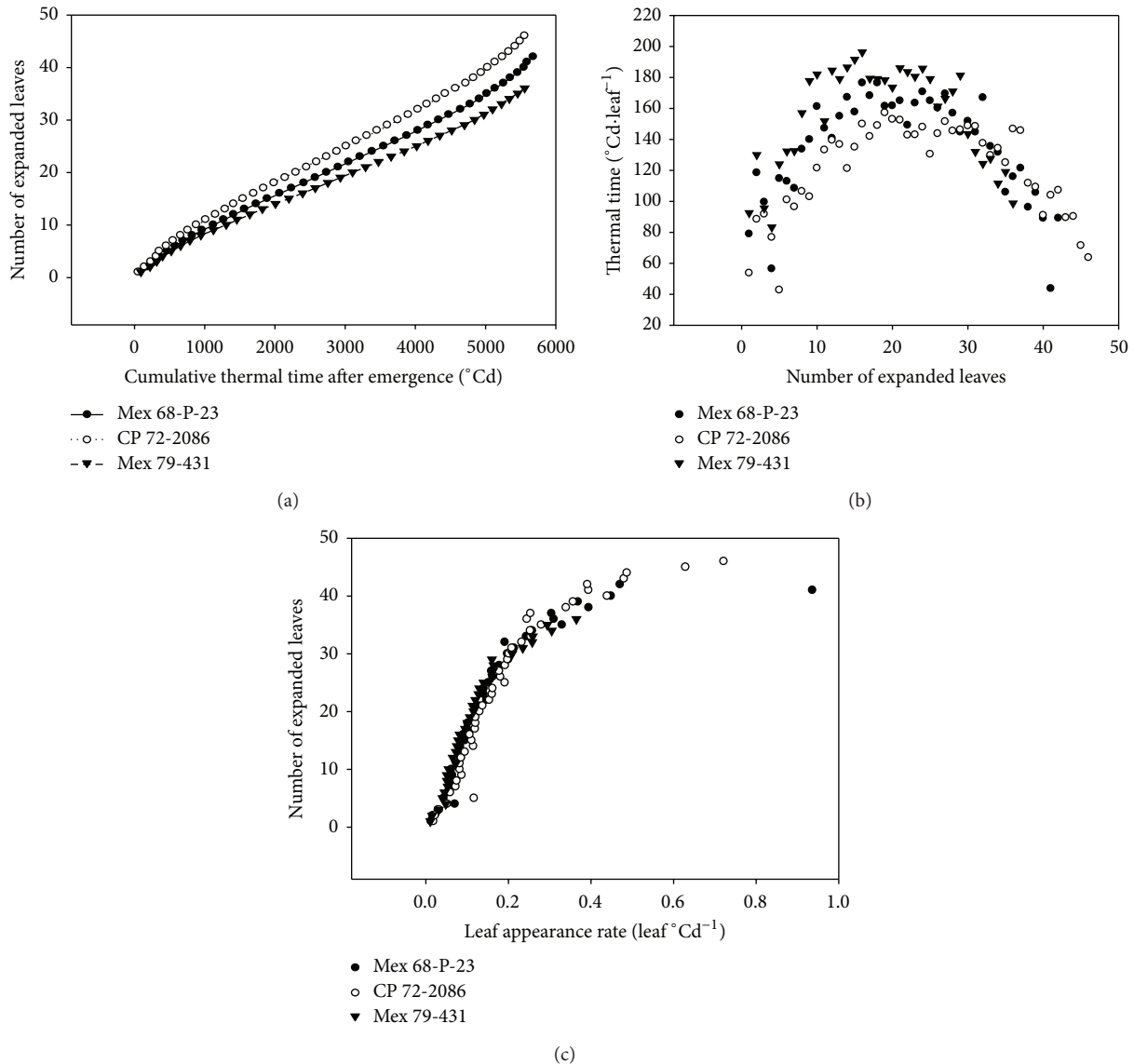


FIGURE 4: The progress of leaf area index with time for three sugarcane cultivars sown on the same date during the period of canopy closure to harvest under high temperature rainfed conditions in Northeastern Mexico.

appear, while leaf #16 required  $148^{\circ}\text{Cd}$  and leaf #40 required  $90^{\circ}\text{Cd}$  (Figure 4(b)).

There were significant differences ( $P < 0.05$ ) between the three cultivars (Table 1) in leaf appearance rate, namely, that canopy development differed, similar to the results observed by Singels and Donaldson [25]. Cultivar Mex 79-431 had the lowest average leaf appearance rate ( $0.099 \text{ leaves}\cdot\text{day}^{-1}$ ) among the three cultivars tested. On the other hand, cultivar CP 72-2086 had the highest rate ( $0.129 \text{ leaves}\cdot\text{day}^{-1}$ ), although its leaf appearance rate was only 14% and 23% greater than Mex 68-P-23 and Mex 79-431, respectively. Our results provide an explanation for the observation that CP 72-2086 showed the most rapid canopy closure in the field; however, the results reported by Bonnett [5] and Inman-Bamber [9] could not be compared with the current results because the base temperature they utilized ( $8^{\circ}\text{C}$  and  $-2$

to  $20^{\circ}\text{C}$ , resp.) was different from that used in this study ( $10^{\circ}\text{C}$ ). These results are consistent with leaf appearance rates reported by Inman-Bamber [9] for the cultivars NCo 376 and N12. At  $5000^{\circ}\text{Cd}$  the number of leaves that had appeared ranged from 32 to 40.

In summary, our results have shown that under rainfed and high temperature conditions, variety Mex 79-431 had the greatest leaf width, the greatest area per leaf, and the greatest estimated yield in tons per acre (data not shown), of the three varieties tested.

#### 4. Conclusions

Genotypic differences were observed in individual leaf area, maximum LAI, total leaf number, and leaf width, resulting in contrasting canopy structures and these differences were

reflected in LAI. Clearly, Mex 79-431 produced a larger leaf area per stalk in the development of the crop than the other varieties tested. This study has shown that future breeding efforts might consider including selection of varieties with leaves of large area during the development cycle as a parameter contributing to high sugarcane yield. Further investigation into the growth and development of sugarcane under high temperatures and water stress and an understanding of the genetic variation of the response could lead to better targeting of varieties for introduction into regions under rainfed conditions that experience high temperatures.

## Competing Interests

The authors declare that they have no competing interests.

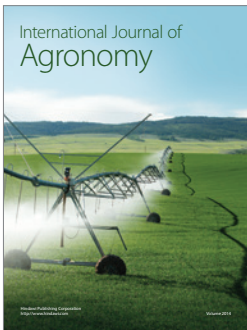
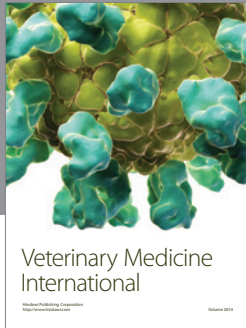
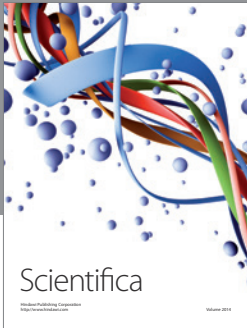
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