

HYDROCYANIC ACID CONTENT AND GROWTH RATE OF SORGHUM X SUDANGRASS HYBRID DURING FALL

Teor de ácido cianídrico e taxa de crescimento do híbrido de sorgo x capim sudão durante o outono

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ABSTRACT

In central Brazil after soybean or other annual agricultural species is harvested, sorghum hybrids are planted in the fall in order to establish pastures for grazing animals. This study conducted for two consecutive years aimed at quantifying the contents of hydrocyanic acid in the leaves and determining plant height, forage dry matter yield and the leaf/stem ratio for sorghum hybrid 1P400 at different ages. Statistical analysis was performed by regression analysis based on plant age. Leaf HCN content decreased with plant growth, ranging from 205.0 and 230.3 mg HCN/100 g leaf DM at two weeks old to 5.9 and 6.1 mg HCN/100 g leaf DM at five weeks old in the first and second year, respectively. The average heights measured varied from 60 and 56 cm, in the 4th week, to 117 and 151 cm, in the 8th week, during the first and second experimental year, respectively. Forage mass increased linearly with age and displayed average of 1.411 and 1.637 kg DM/ha in the first year and, 2.905 and 3.640 kg DM/ha in the second year, during the 7th and 8th week, respectively. Leaf proportion decreased while stem increased linearly with plant age. The leaf/stem ratio decreased with plant growth, elongation and increasing stem weight. The sorghum hybrid should be grazed only after five weeks or when the plant height is above 80 cm, in order to avoid the risk of cyanide poisoning.

Index terms: Annual grasses, cyanogenic plants, micro-sodium test, toxic plant.

RESUMO

No Brasil Central, híbridos de sorgo podem ser semeados no outono, após a colheita da soja ou outra espécie de planta anual, com o objetivo de fornecer alimento aos ruminantes por meio de pastejo. O trabalho foi desenvolvido, por dois anos consecutivos, com o objetivo de quantificar os teores de ácido cianídrico das folhas e mensurar a altura das plantas, produção de massa seca de forragem e a proporção de folha e colmo nas diferentes idades do híbrido de sorgo IP400. A análise estatística foi realizada por meio de análise de regressão em função da idade das plantas. O teor de HCN nas folhas decresceu com o desenvolvimento das plantas, apresentando 205,0 e 230,3 mg HCN/100 g de MS de folha, com duas semanas de crescimento e 5,9 e 6,1 mg HCN/100 g de MS de folha, na quinta semana de crescimento, no primeiro e segundo ano, respectivamente. As plantas apresentaram em média 60 e 56 cm, na 4^a semana, e 117 e 151 cm na 8^a semana, no primeiro e segundo ano, respectivamente. A massa de forragem aumentou linearmente, com a idade, apresentando, em média, na sétima e oitava semanas de avaliação, 1.411 e 1.637 kg de MS/ha no primeiro ano e 2.905 e 3.640 kg de MS/ha no segundo ano, respectivamente. Com o crescimento das plantas, a proporção de folhas diminuiu e a proporção de colmo aumentou linearmente. A relação folha/colmo diminuiu com o crescimento, com o alongamento das plantas e aumento do peso dos colmos. O híbrido de sorgo deve ser pastejado, somente após a quinta semana de crescimento ou quando as plantas tiverem acima de 80 cm para não haver risco de intoxicação por HCN.

Termos para indexação: Gramíneas anuais, planta cianogênica, teste micro-sódico, planta tóxica.

(Received in may 28, 2013 and approved in july 5, 2013)

INTRODUCTION

In tropical regions, such as central Brazil, sorghum hybrids can be planted off-season in February or March (fall), after soybean or other annual species is harvested, in order to provide feed to grazing ruminants. On the other

hand, young sorghum plants are considered cyanogenic because they contain cyanogenic glycoside, esters that can release toxic substances when the plant structure breaks due to stress caused by grazing, trampling or drought (GILLINGHAM, 1969; MELO, 2003; MONTAGNER, 2005).

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Dhurrin, the most important cyanogenic glucoside, in the presence of the enzyme β -glucosidase releases sugar and hydrocyanic acid (HCN), a colourless very volatile liquid, considered one of the most toxic substances ever known. Ruminants are more susceptible to HCN intoxication compared to monogastric. The acidic stomach pH of monogastrics do not allow the enzyme linamarase to act and, therefore, the cyanide release slows down, allowing time for its elimination without reaching the lethal dose (DOWLING; MACKENZIE, 1993). However, in ruminants the combination of neutral pH and the presence of bacteria capable of hydrolysing linamarina are considered high risk since cyanide is released rapidly and may reach the lethal dose before being eliminated from the animal organism. Haque et al. (2002) also studied pH effect and concluded that HCN is more toxic in neutral pH.

Nóbrega Junior. et al. (2006) investigated HCN poisoning in goats feeding on *Sorghum halepense* (L.) Pers., a highly toxic and invasive species. The goats, after 30 days, showed severe dyspnoea and frequent urination, sign of acute poisoning that lead to death.

Several sorghum varieties and hybrids with different HCN potential in their leaves have been studied, and a strong genetic effect/component was detected on the HCN content in the plants (LAMB et al., 1991). Wheeler et al. (1990) studied the hybrids *S. bicolor* (L.) Moench and *S. sudanense* (Piper) Stapf and reported strong influence of nitrogen fertilization and plant age on the HCN contents in the leaves. The regrowth forage of Zulu hybrid, after three weeks, had 100 mg HCN/100 g DM when fertilized with 200 kg N/ha and 76 mg HCN/100 g DM without topdressing while the hybrid Silk had 185 mg HCN/100 g DM when fertilized with 200 kg N/ha and 33 mg HCN/100 g DM without topdressing, thus showing different behaviour for different hybrids. Fertilization with phosphate did not affect HCN levels while concentrations of HCN dropped sharply with age, plant height and number of expanded leaves.

An important factor for the accumulation of HCN is the rapid plant regrowth after the first rains, which can be compounded by a period of rapid growth after slow growth periods due to drought or low temperatures (GORASHI; DROLSOM; SCHOLL, 1980; RADOSTITS, 2002).

Even for different regions and assessment times, the sorghum hybrid shows flexibility regarding planting season and good forage yield. Simili et al. (2010) studied irrigated sorghum AG 2501C and reported 4 grazing cycles with an average yield of 2.800 kg DM/ha per cut, from April to September, 2002. Another study with two sowings (December and March)

had similar yields for the sorghum hybrid 1P400, 3.234 and 3.135 kg DM/ha per cutting, respectively (SIMILI et al., 2011).

It is necessary to establish whether the sorghum hybrid 1P400 poses the risk of cyanide poisoning for grazing cattle and the ideal plant age when grazing provides good forage for the animals and the risk of poisoning is not present any longer. Studies of this nature are of great importance for cattle, sheep and goats due to the increasing use of this grass for grazing in various tropical regions.

The aim of this study was to quantify the levels of hydrocyanic acid in the leaves, to determine plant height, forage dry matter yield and the leaf/stem ratio at different harvesting dates of hybrid sorghum 1P400 to establish the ideal time to start grazing by cattle.

MATERIAL AND METHODS

The experiment was conducted for two consecutive years at the experimental farm of the Agência Paulista de Tecnologia dos Agronegócios (APTA) in Ribeirão Preto, mid-east region of Sao Paulo state (21°42'S, 47°24'W and altitude 535 m). The climate is tropical with dry winter. High and low temperatures, as well as rainfall in the region, are shown in figure 1.

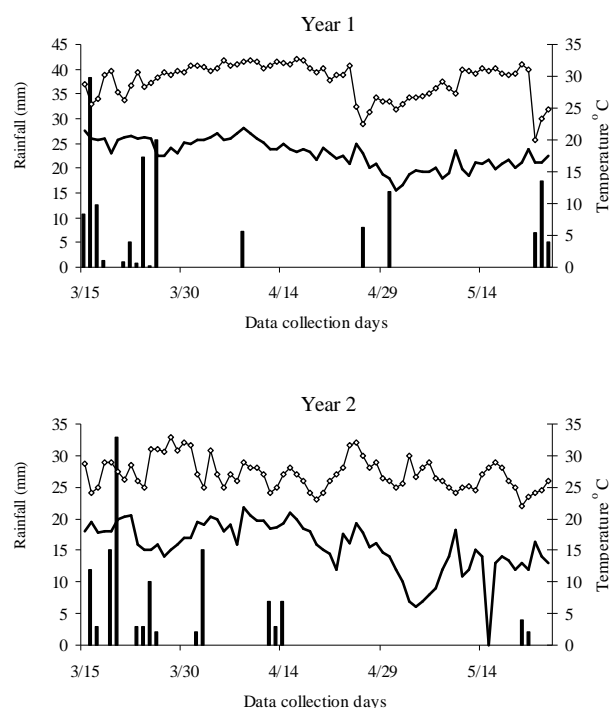


Figure 1 – Rainfall and maximum and minimum temperatures during the two experimental years in Ribeirão Preto, São Paulo, Brazil.

The area has a slightly undulating relief and the soil is classified as Dystroferric Red Latosol (EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA-EMBRAPA, 1999). The sorghum was planted in a 600 m² area divided into three plots of 200 m² (4 x 50) each, with two repetitions per plot. The chemical characteristics of soil samples collected from every plot were: plot 1, pH CaCl₂ = 4.9; organic matter = 43 g/dm³; phosphorus in resin = 38 mg/dm³; sum of bases = 48.9 mmol/dm³; and base saturation, V% = 51%. Soil samples from plot 2 displayed CaCl₂ = 4.6; organic matter = 37 g/dm³; phosphorus in resin = 30 mg/dm³; sum of bases = 42.1 mmol/dm³; and V% = 42% while for plot 3, pH CaCl₂ = 4.9; organic matter = 40 g/dm³; phosphorus in resin = 21 mg/dm³; sum of basis = 45.4 mmol/dm³; and V% = 49%.

The weeds were desiccated using glyphosate herbicide before sowing. Sowing was performed on March 20 in both experimental years, under the no-tillage system, using 12 kg seed/ha of the sorghum hybrid (*S. bicolor* x *S. sudanense*) 1P400, by Dow Agrosiences, recommended for grazing. Fertilization used 120 kg/ha of 8-28-16+Zn fertilizer, without topdressing since there was residual fertilization of the soybean culture planted earlier in both experimental years.

Plant sampling was random within each plot and consisted of cutting a meter of grass at ground level for each repetition, two per plot. The samples were then taken to the laboratory where they were weighed and separated according to each response variable. The response variables studied were: hydrocyanic acid content of the leaves, forage dry matter yield (FDM), plant height, proportion of leaves and stems and leaf/stem ratio (L/S).

The first samples were collected at the end of the second growth week, so the plants were 14 days old and continued until the inflorescences emerged in the eighth week.

The hydrocyanic acid content was measured in the leaves until values were close to zero, that is, until the 5th week for both experimental years. The HCN content was determined by the Guignard test (MONTGOMERY, 1969), which is a semi-analytical quantifying testing where the resulting colours are compared to a standard. Sodium picrate paper is prepared by dipping the filter paper ($\pm 1 \times 10$ cm) into a picric acid and sodium carbonate solution. The 1P400 sorghum hybrid leaves were separated into leaf blades and stems (with sheath), chopped and weighed into ± 0.5 -g samples that were then placed into lidded test tubes (2 x 12 cm) and added water (± 1 mL). The sodium picrate filter papers were suspended into the test tubes from the lid, which were taken to a water bath at 38°C, for

at least 12h. The result was considered positive when the colour of the sodium picrate paper strip changed from yellow to earthy red. Subsequently, the sorghum hybrid 1P400 paper strips were compared to a standard curve prepared for a potassium cyanide solution containing 0 to 1 mg cyanide per mL. The amount of hydrocyanic acid was then determined over time (in weeks) by calculating the HCN per gram of leaf dry matter.

Forage dry matter was obtained from the subsample containing the plant tops that were dried in a forced air drying oven at 55°C, for at least 72 hours until constant weight (SILVA; QUEIROZ, 2002).

Plant height was determined in ten sampling points per meter using a cm-graduated ruler placed at the leaf inflection point or the tip of the flag leaf, when there was inflorescence.

The different forage fractions were obtained from the subsamples that were separated into leaf (leaf blade) and stem (with sheath). These fractions were also dried in a forced air drying oven at 55°C, for at least 72 h until constant weight to determine dry matter content. The leaf/stem ratio was obtained by dividing leaf dry matter and stem plus sheath dry matter.

Statistical analysis was performed by regression analysis based on plant age, using the software PROC GLM (STATISTICAL ANALYSIS SYSTEM – SAS, 2003) for each experimental year separately.

RESULTS AND DISCUSSION

During the first experimental year, leaf HCN content decreased with aging plants (Figure 2). The values ranged from 205 to 5.9 mg HCN/100 g leaf dry matter for two- and 5-week old plants, respectively. In the second year, the hydrocyanic acid curve behaved differently (Figure 2) since HCN content increased until the fourth week, with average 230.3 mg HCN/100 g leaf DM, and decreased rapidly until the fifth week, when the plants reached average 6.1 mg HCN/100 g leaf DM (Figure 2).

Haque et al. (2002), also studied HCN content of sorghum leaves (*S. vulgare* Pers.) in Australia and reported at the end of the 2nd, 3rd and 4th growth weeks the following toxic levels of HCN: 280, 40 and 60 ppm HCN in fresh leaves and, therefore, they recommended that the livestock producers should monitor carefully the grazing activity.

Levels between 75 and 100 mg HCN per 100 grams of leaf dry matter poses a poisoning danger, when the leaves are ingested by ruminants according to Wall and Ross (1975). The greater values found in the present study

point out towards potential poisoning risk in case the sorghum hybrid IP400 is consumed by the cattle during this period.

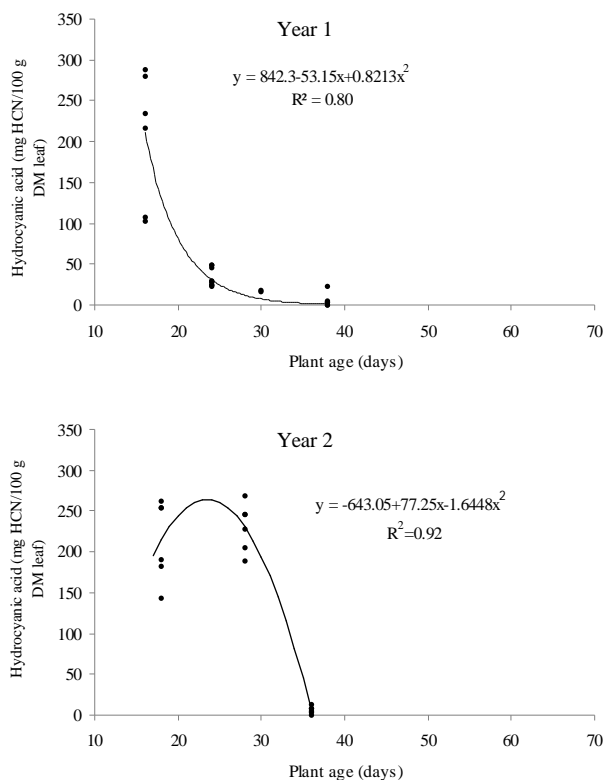


Figure 2 – Hydrocyanic content (mg HCN/100g leaf dry matter) of the hybrid *Sorghum bicolor* x *Sorghum sudanense* 1P400 assessed in two experimental years in Ribeirão Preto, São Paulo, Brazil.

A greenhouse experiment, where the plants were cultivated under controlled temperature at 30°C and 20°C during the day and night, respectively, and another where the temperatures were changed to 20°C and 10°C during the day and night, respectively showed that HCN contents were higher in younger leaves and increased significantly at lower temperatures (GORASHI et al., 1980). This study corroborates this trend towards lower temperatures. During the second experimental year, when the temperatures were lower, the poisoning risk was present until the 5th week (35 day old plants) when plant height was 80 cm. During the growth period between 14 and 28 days (from April 3 to 17), in the second year, the temperatures fluctuated between 16 and 20°C (Figure 1) while in the first year, at the same time, the temperatures

fluctuated between 20 and 32°C (Figure 1). In the latter, when the grass was only 48 cm tall at 21 days old there was no poisoning risk and the grass was safe to grazing as early as the 3rd week. Mulcahy et al. (1992) investigated some sorghum varieties for grazing and also reported large difference for HCN levels depending on the period, in the first year, the average was 570 mg/kg DM while in the second year, the average was 123 mg/kg DM.

Figure 3 shows plant height versus time (age). For both experimental years, plant height was close to 50 cm at the end of the 4th week (60 cm and 56 cm average in the first and second year, respectively). During the second year, the grass displayed fast growth from the 5th week until the end of the evaluation period and final height was bigger than in the first year. The sorghum hybrid 1P400 reached heights of 117 and 151 cm at the end of the 8th week, in the first and second year, respectively.

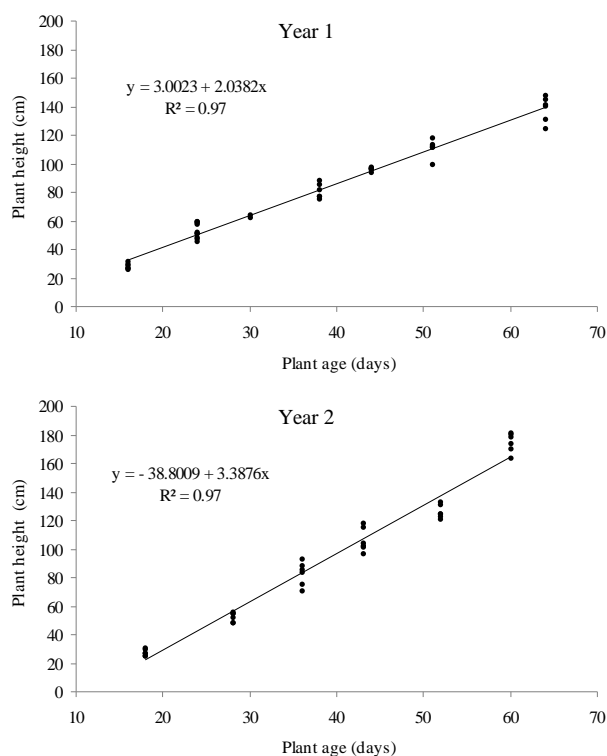


Figure 3 – Plant height of the hybrid *Sorghum bicolor* x *Sorghum sudanense* 1P400 determined for two experimental years in Ribeirão Preto, São Paulo, Brazil.

Melo et al. (2003) while investigating the sorghum hybrid AG2501C reported a height of 105 cm for a 50-

day old plant, which was sowed in November in a crop area in Rio Grande do Sul, similar to the result reported in this study.

Herbage mass increased linearly with sampling time for both years (Figure 4); furthermore, in the second year, the higher rainfall recorded at the beginning of the year (Figure 1) resulted in even higher herbage mass. The rainfall accumulated during the first 30 days of culture was 62.1 and 100 mm in the first and second year, respectively.

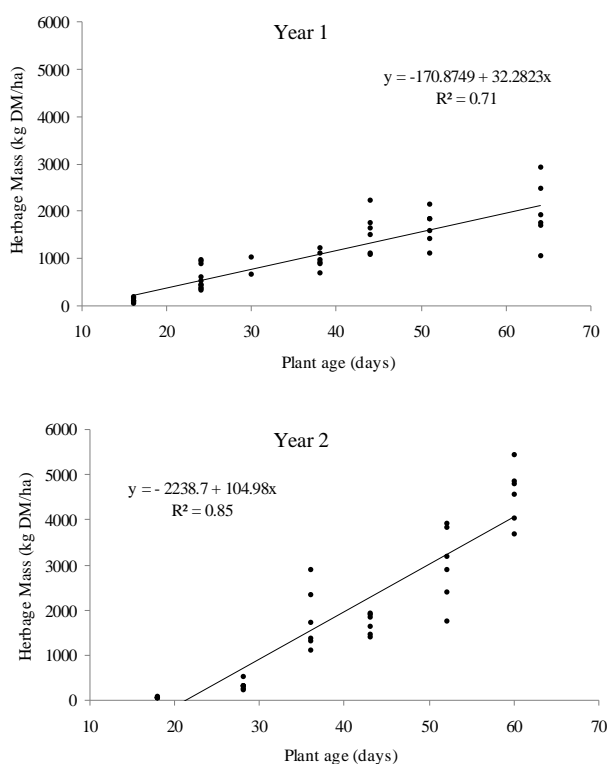


Figure 4 – Herbage dry matter of the hybrid *Sorghum bicolor* x *Sorghum sudanense* 1P400 assessed in two experimental years in Ribeirão Preto, São Paulo, Brazil.

DM/ha in the first year and, 2905 and 3640 kg DM/ha in the second year, respectively, showing that rainfall strongly influenced plant growth rate during the fall.

Montagner et al. (2005) studied eight varieties of sorghum hybrid cultivated in Rio Grande do Sul, from December to January and reported dry matter yield between 700 and 1580 kg/ha, for 35 and 40-day old sorghum, similar to the values found in this study for 2005.

Mello et al. (2003) studied the sorghum hybrid AG2501C and reported an yield of 1770 kg DM/ha after

50 days of growth, similar to the value found in the first year of this study, on the other hand, Simili et al. (2011) found for the sorghum hybrid 1P400, dry matter yields of 3234 and 3135 kg/ha per cutting for an irrigated crop, values closer to the results of the second year of this study.

Figures 5 and 6 show the fractions of leaves and stem. The leaf fraction decreased with the growth rate (Figure 5) while the ratio stem/sheath increased linearly (Figure 6). The hybrid sorghum grass displays cespitose growth habit with a great ability to grow stems, which despite being nutritious, are not efficiently consumed when their height exceeds 1.20 m in grazing systems.

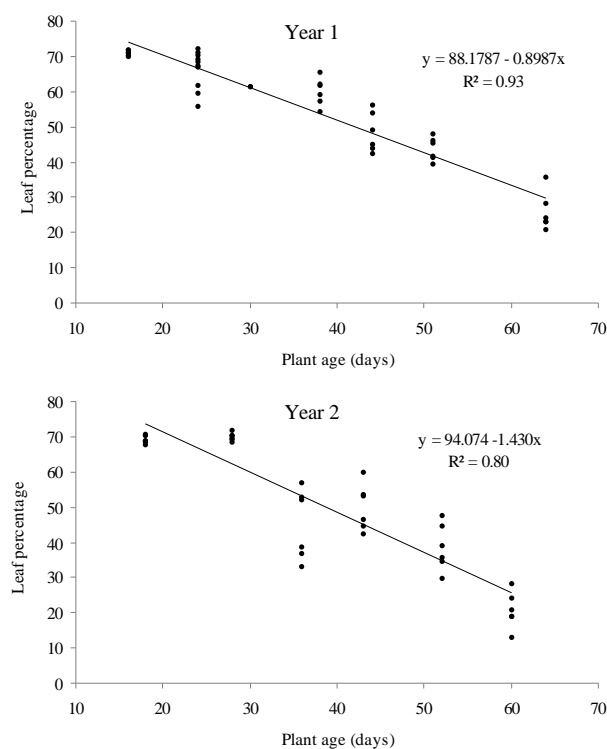


Figure 5 – Percentage of dry leaves of the hybrid *Sorghum bicolor* x *Sorghum sudanense* 1P400 assessed in two experimental years in Ribeirão Preto, São Paulo, Brazil.

The plants reached the height of 90 cm in the sixth and fifth week in the first and second year, respectively, which represented 50% leaf fraction.

Melo et al. (2003) reported 52.5% of leaves after 50 days for the sorghum hybrid AG 2501C, in Rio Grande do Sul, when the plant height was 105 cm, different from the values found in this study.

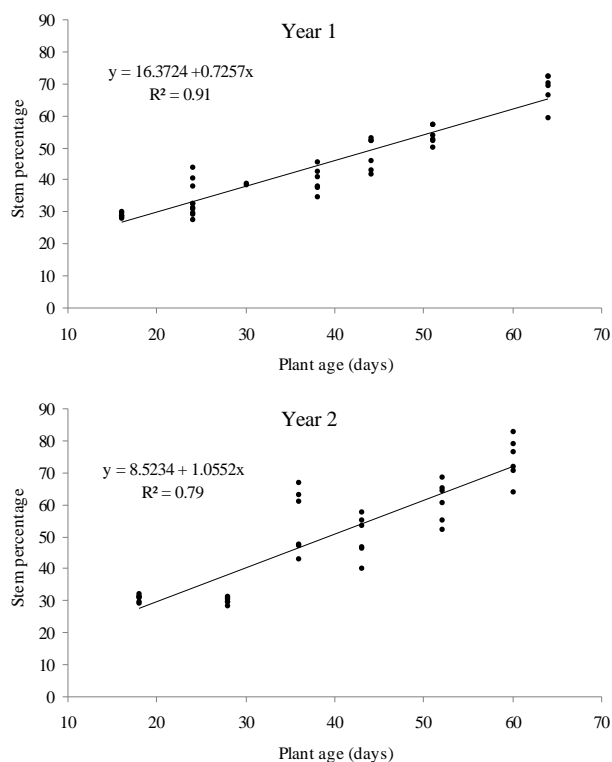


Figure 6- Percentage of dry stem (with sheath) of the hybrid *Sorghum bicolor* x *Sorghum sudanense* 1P400 assessed in two experimental years in Ribeirão Preto, São Paulo, Brazil

The leaf/stem ratio decreased significantly from the fifth week of growth (Figure 7) due to elongation and consequent weight increase, as it is characteristic of sorghum plants. Mulcahy et al. (1992) studied some varieties of sorghum as a herbage and reported a leaf/stem ratio ranging from 1.11 to 1.50 for plant heights between 90 and 95 cm, respectively, higher and better values compared to this study.

The leaf/stem ratio is an important characteristic of canopy structure mainly regarding tropical grasses that present rapid stem development (STOBBS, 1973; SILVA; GOMIDE, 1994). This characteristic can influence animal grazing behaviour (STOBBS, 1973) and their performance as well (SILVA; GOMIDE, 1994; EUCLIDES, 1999). Accordingly, Stobbs (1973) showed that longer grazing intervals are associated with higher total biomass density but generally lower leaf density. Thus, stem elongation despite intensifying herbage accumulation, compromises canopy structure decreasing the leaf/stem ratio and sorghum intake by the animals.

In this context, the sorghum hybrid should be grazed when height is between 90 and 100 cm, the leaf/stem ratio varies between 1.5 and 0.8 and there is no

poisoning risk by HCN. However, the time necessary to reach this height depends on the amount of water available in the soil for plant growth.

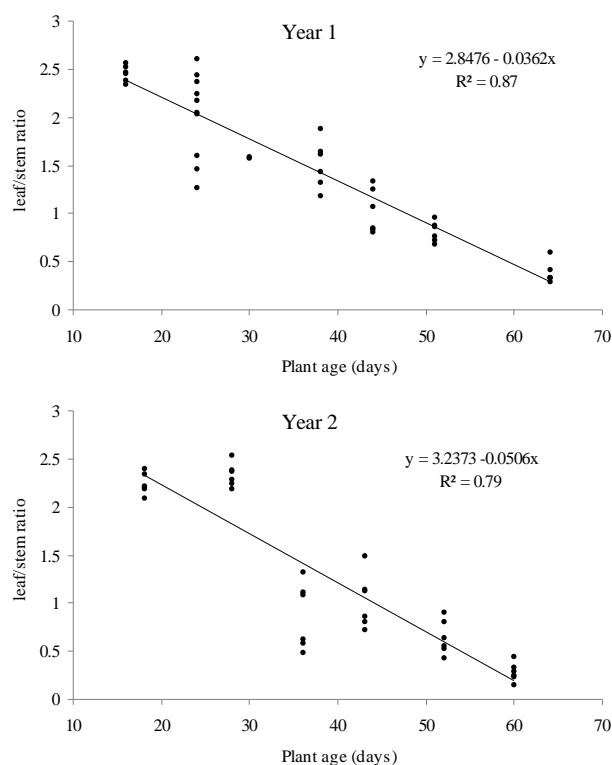


Figure 7 – Leaf/stem ratio of the hybrid *Sorghum bicolor* x *Sorghum sudanense* 1P400 assessed in two experimental years in Ribeirão Preto, São Paulo, Brazil.

CONCLUSIONS

Animal poisoning risk by HCN exists when the sorghum hybrid is young and, therefore, should not be consumed when height is less than 80 cm. In the fall culture, the best pre-grazing height of sorghum 1P400 is between 90 and 100 cm, when leaf/stem ratio is higher and there is no risk of poisoning any longer.

ACKNOWLEDGEMENTS

This study was financed by FAPESP (grant number 2004/13427-3)

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