Stem characteristics of two forage maize (*Zea mays* L.) cultivars varying in whole plant digestibility. I. Relevant morphological parameters

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Abstract

The morphology and rumen fermentation kinetics of the maize cultivars (*Zea mays* L.) Vitaro and Volens were investigated in detail throughout their growing period as a first step towards understanding the relation between plant characteristics and cell wall fermentability of forage maize. Vitaro is known to have a 9% higher whole plant organic matter digestibility than Volens. Leaf and internode development, fresh (FW) and dry weight (DW) per plant and dry matter content (DMC) of leaves, internodes and developing ears, as well as rumen fermentation characteristics of the stem, were monitored during two seasons. Vitaro plants had a larger final leaf area than Volens plants but their number of leaves (and internodes) was the same. Fully developed Vitaro internodes were shorter and thicker than Volens internodes, resulting in a shorter plant for Vitaro. After anthesis, FW and DW of individual internodes did not vary significantly throughout the growing period. Whole plant FW increased sharply after anthesis, which was associated with the development of the main ear. In both cultivars, DMC of the whole plant more or less doubled between anthesis and harvesting. Vitaro had a higher DW per plant than Volens, but not a larger ear proportion. In rumen fermentation tests on whole stem samples, using the gas production technique, gas production after 72 hours and Tilley & Terry digestibility were significantly higher for Vitaro than for Volens, indicating a higher total degradability for Vitaro. It was concluded that the two cultivars form a suitable model system for studying the causes of differences in rumen fermentation between maize roughages.

Additional keywords: development, morphology, fermentation kinetics

Introduction

The feeding value of roughages in ruminants largely depends on the rate and extent of their fermentation in the rumen. Plant characteristics that influence the fermentation process are not fully known or understood. Physical and chemical properties of the cell wall, tissue anatomical structure, and particle size in the rumen are assumed to influence fermentation. Moreover, physical structure of the cell wall partly determines its accessibility for rumen microbes (Wilson, 1993).

Forage maize (*Zea mays* L.) is important roughage for ruminants. However, maize stems have a relatively low fermentability, partly caused by the high lignin content of the cell walls, which increases with the crop's age (Jung, 1989). A better understanding of maize cell wall fermentation could be instrumental to increase the feed value of maize roughage.

To investigate the factors influencing cell wall fermentability of maize, it seemed necessary to characterize plant and stem morphology of cultivars differing in cell wall fermentability to see whether any fermentation characteristics could be related to morphological characteristics that are recognizable in the field and can be easily quantified. Such a relation would be of great practical use.

In this paper, the forage maize cultivars Vitaro and Volens were studied in detail throughout their growing period. Whole plant organic matter digestibility of Vitaro (as measured with Near Infrared Reflectance Spectroscopy) is known to be 9% higher than that of Volens (Advanta Seeds BV; unpublished results). Differences in stem digestibility probably are considerably larger. Through *in vitro* gas production fermentation it is possible to discriminate between fermentation of the cell content and fermentation of the cell wall (Groot *et al.*, 1996). Fermentation of cell walls is usually low, thus limiting whole plant fermentation, whereas fermentation of cell contents is usually rapid and complete.

The two cultivars may be a suitable model system for studying fermentability of maize roughages in the rumen, and were therefore used to investigate the relation between anatomy, chemistry and fermentability in a larger systematic study of fermentation within a selected internode, among internodes at a selected time, and within a selected internode throughout the growing period. This paper introduces the growth of the two maize cultivars and their fermentability in general terms. Subsequent papers will present details on the differences in anatomy, chemical composition and fermentability within internodes, over internodes within a plant and over time of one internode.

Materials and methods

Field experiments and plant observations

The maize cultivars Vitaro and Volens were grown in 1999 and 2000 on a heavy river clay soil in Wageningen, in 18 × 20 m plots at a density of 100,000 plants per ha. Vitaro and Volens are commercially grown cultivars in the Netherlands (Anon., 2002) and France (Anon., 1999), respectively.

In 1999, the maize was sown on 19 May and both cultivars started flowering in the first week of August. Uniform plants were selected to monitor leaf production and development. Samples of internode 7 were taken on 9, 12, 14, 16, 19, 22, 26 and 29 July, 2, 9, 16, 23 and 30 August and 13 and 27 September. Internode 7 was chosen because it had a high dry weight (reducing sample size), was not accompanied by an ear, and had few adventitious roots, thus avoiding complications with the interpretation of the data. With Vitaro, internode 7 was the second internode above the soil surface, with Volens the third. Whole stem samples were taken on 2 August 1999, when both cultivars showed anthesis.

In 2000, the maize was sown on 3 May, and both cultivars started to shed pollen in the first week of August. Samples of internode 7 were taken from uniform plants on 26 June, 3, 7, 10, 13, 17, 20, 24 and 27 July, 3, 10, 14, and 21 August and 4 and 18 September. Whole stem samples were taken on 3 August, when both cultivars were flowering. To identify internode 7 and to check for similarity between the two seasons, the length of leaves 6, 7 and 8 was measured.

In 1999, the rate of leaf appearance was monitored by regularly counting the number of fully-grown leaves. Internodes were numbered from the base of the stem. The internode accompanying leaf 1 was designated internode 1. Internode number was verified by measuring the length of the leaf accompanying the internode (Bos *et al.*, 2000). The leaf area of individual fully-grown leaves was calculated by means of leaf length and maximum leaf width, using the equation:

$$k \times \text{maximum leaf width} \times \text{leaf length}$$

where k is the leaf shape factor. Bos *et al.* (2000) showed that for maize leaves 3 to 8 this factor lies between 0.67 and 0.71. In our study, k was set at 0.70 for all leaves. The internode and stem samples were stored at –20°C until further analysis.

Temperature sum

The development of the maize plant is closely related to temperature. To be able to compare the growing seasons of 1999 (relatively dry and warm) and 2000 (relatively wet and cool), results were related to the temperature sum (T_{sum}; °C.d). This temperature sum was calculated from the daily average temperatures minus the base temperature, added over the period from sowing, for which the following equation was used (Sibma, 1987):

$$T_{sum} = \Sigma[(T_{max} + T_{min})/2 - T_{base}]$$

where
T_{max} = daily maximum temperature (°C);
T_{min} = daily minimum temperature (°C);
T_{base} = 10 °C.

Plant weights

For the determination of fresh weight (FW) and dry weight (DW) of individual plant parts two or more plants per cultivar were dissected into leaf laminae, leaf sheaths, ears, internodes and tassels. For plant samples taken after August, also grain weight was determined. DW was determined after drying for at least 48 hours at 70 °C. For chemical and *in vitro* analyses, the samples were ground to pass a 1 mm sieve.

Fermentation

Fermentation characteristics of whole stem samples (without ears and leaves) taken at anthesis and at the end of the growing period (ensiling stage; 27 September 1999 and 9 October 2000), were determined using the gas production technique as described by Cone *et al.* (1996).

The gas production profiles obtained were fitted with a three-phase model describing the fermentation of the soluble components (subcurve 1), the non-solubles (subcurve 2) and the microbial turnover (subcurve 3) (Cone *et al.*, 1997). Each subcurve is described by the parameters a (asymptotic maximum gas production in ml per g organic matter), b (time in hours to reach 50% of a) and c (factor determining the steepness of the curve) (Groot *et al.*, 1996). The subnumbers of a, b and c in this paper refer to the number of the subcurve. The NLREG package (Sherrod, 1995) was used to carry out the non-linear regression analyses.

The gas production after 3 hours (GP3) was defined as a1 and the gas production between 3 and 20 hours (GP20–GP3) as a2. These values indicate the fermentation rates of the readily digestible fractions in the organic matter. Some cell wall material is fermented at a much slower rate.

An *in vitro* assay was carried out on whole stems without ears and leaves, using the Tilley & Terry (T&T) technique (Tilley & Terry, 1963).

Statistical methods

Statistical significance was calculated using Student's t-test or a general analysis of variance, with Statistix for Windows version 2.0. Experimental units consisted of 2 to 4 plants.

Results

Leaf development

In 1999, the day leaf number 0 emerged – taken as a prediction of emergence date and determined by extrapolation in Figure 1 – was at a T_{sum} of about 40 °C.d for both cultivars. The first fully-grown leaf was observed at the end of May at a temperature sum of 62 °C.d (Figure 1). Leaf appearance was linearly correlated with T_{sum} ($R^2 = 0.99$ for both cultivars; $n = 14$), with an average leaf appearance rate of about 0.03 leaves

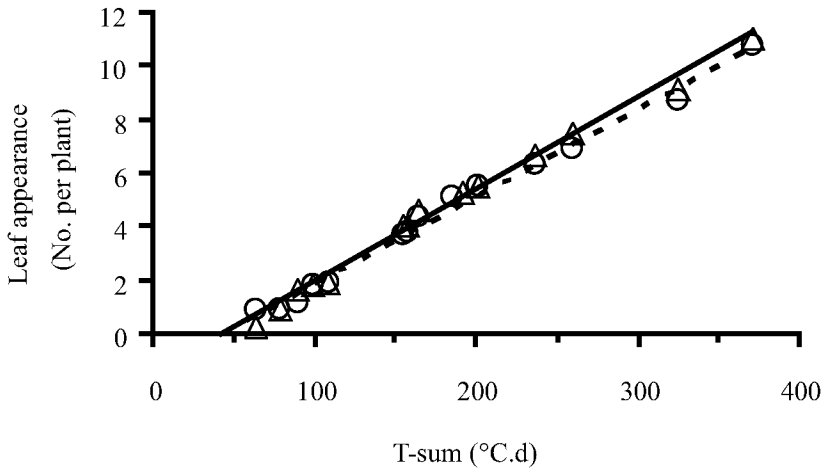

Figure 1. Relationship between leaf appearance (by leaf number) of Vitaro and Volens maize plants and temperature sum after sowing (T-sum) in the growing season of 1999. -- △ -- Vitaro, — ○— Volens; lines indicate fitted curves).

per °C.d for both cultivars. The rate of leaf appearance of Vitaro was slightly higher: to produce 10 fully-grown leaves Vitaro needed 5 °C.d less than Volens. (The emergence of leaves 12 to 16 was not recorded.) Both cultivars produced a total of 16 leaves on average and flowered in the first week of August. However, in Vitaro the T_{sum} until the onset of anthesis was 5 °C.d less than in Volens. In 2000, leaf appearance was not recorded.

In both years, leaves 6 to 8 were longer for Volens than for Vitaro. The length of leaves 7 and 8 of Vitaro was slightly ($P > 0.05$) shorter in 2000 than in 1999 (Table 1). But as the difference in length between individual leaves was at least 10 cm, this did not compromise internode identification. In 2000, leaf 6 of Vitaro and leaves 6 to 8 of Volens did not differ significantly ($P < 0.05$) in length from the same leaves in 1999.

Table 1. Average length (n = 13–45) of the fully-grown leaves 6, 7 and 8 of Vitaro and Volens maize plants in the growing seasons of 1999 and 2000, with standard errors of the means (n = 13–45).

Cultivar	1999			2000		
	Leaf 6	Leaf 7	Leaf 8	Leaf 6	Leaf 7	Leaf 8
	(cm)					
Vitaro	39 ± 0.8f[1]	55 ± 1.2de	70 ± 1.0b	41 ± 0.5f	52 ± 0.7d	66 ± 0.9bc
Volens	49 ± 0.9e	64 ± 1.0c	78 ± 0.6a	51 ± 0.8e	64 ± 0.9c	77 ± 0.9a

[1] Averages within and across rows, followed by a different letter are statistically different ($P < 0.05$).

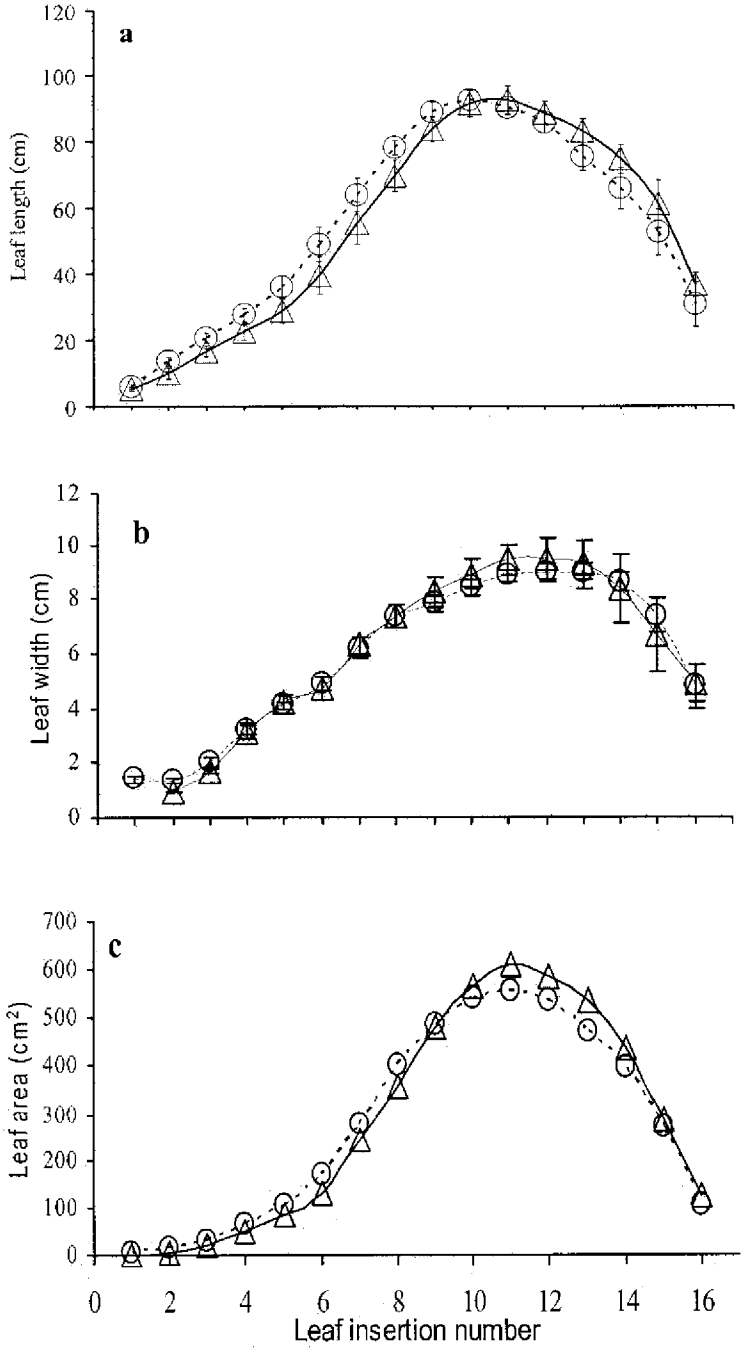

Figure 2. Leaf length (a), leaf width (b) and leaf area (c) versus leaf insertion number of Vit Volens maize plants in 1999. (—△—Vitaro, - - - ○ - - - Volens)

Table 2. Average length (cm; n = 49–62) of internode 7 of Vitaro and Volens maize stems during the growing season of 2000, with standard errors of the means, and temperature sum (T_{sum}) since the date of sowing.

	Date				
	26 June	29 June	3 July	7 July	10 July
Cultivar					
Vitaro	9.4 ± 0.6d[1]	13.8 ± 0.4bc	15.6 ± 0.3b	15.6 ± 0.3b	15.6 ± 0.3b
Volens	7.7 ± 0.5d	13.2 ± 0.5c	21.5 ± 0.3a	23.3 ± 0.2a	22.9 ± 0.3a
T_{sum} (°C.d)	295	300	324	349	359

[1] Averages within and across rows, followed by a different letter are statistically different ($P < 0.05$).

Leaf area

The area of each leaf was calculated in 1999, using length and width measured when the particular leaf was fully grown and not yet senescent. During the development of the plant, lower leaves gradually senesced, starting with leaf 1 and slowly progressing to subsequent leaves. By the end of September, the lower 6 leaves had senesced in many plants.

Leaves 1 to 9 of Vitaro were significantly shorter than the same leaves of Volens, whereas leaves 12 to 16 were significantly longer. The length of leaves 10 and 11 did not differ statistically between the two cultivars.

Leaves 2 and 3 of Vitaro were narrower, leaves 4 to 8 had about the same width as the corresponding leaves of Volens (difference smaller than 2 mm), and leaves 9 to 13 were wider than those of Volens. Leaves 14 to 16 were narrower or had a similar width as those of Volens. Only the differences in width between the two cultivars for leaves 3, 9 and 11 were statistically significant.

The area of the leaves at and immediately above the ear (leaf insertion numbers 10 to 16) was larger for Vitaro than for Volens (Figure 2C).

Internode elongation and stem dimensions

In 2000, elongation of internode 7 was complete by 3 July for both cultivars (Table 2). Although there was a statistically significant year × cultivar interaction, in both years the final length of internode 7 was significantly greater for Volens than for Vitaro (Tables 2 and 3), whereas its diameter was significantly smaller (Table 3).

As for plant height there was a statistically significant cultivar effect ($P < 0.001$), a statistically significant year effect ($P < 0.001$) and a statistically significant year × cultivar interaction ($P = 0.006$). Vitaro plants were statistically shorter than Volens plants ($P < 0.001$), and plants were statistically shorter in 1999 than in 2000 ($P < 0.001$), but

Table 3. Average length and diameter of internode 7, and average height of the whole plant measured at growing seasons of 1999 and 2000, with standard errors of the means.

	1999		2000	
	Vitaro	Volens	Vitaro	Volens
	(cm)			
Internode 7				
Length	16 ± 0.4	22 ± 0.2	16 ± 0.3	23 ± 0.3
Diameter	2.6 ± 0.04	2.4 ± 0.03	3.0 ± 0.04	2.5 ± 0.03
	(n = 45)		(n = 50)	
Whole plant				
Height	225 ± 3.5	270 ± 2.1	264 ± 4.1	292 ± 2.2
	(n = 10)		(n = 20)	

the difference between the two cultivars was smaller in 2000 than in 1999. This cultivar effect was reflected in the length of internode 7.

Plant growth and development

In both 1999 and 2000, the plants flowered (anthesis) in the first week of August. The temperature sum at the onset of anthesis was 483 °C.d in 1999 and 503 °C.d in 2000. During the first 200 °C.d after anthesis, internode 7 varied little in dry weight (Figure 3), but during the final phase of the growing period there was both a considerable cultivar × year interaction ($P = 0.0066$) and a year effect ($P = 0.0007$).

Whole plant fresh weight was higher for Vitaro than for Volens, but fresh weight changed little after anthesis. The average FW of Vitaro over all samples was 775 g, against 636 g for Volens. At the last sampling date in 1999, Vitaro FW had dropped slightly below that of Volens (data not shown).

Between anthesis (T_{sum} 483 °C.d in 1999, and 503 °C.d in 2000) and the last sampling date (T_{sum} 934 °C.d in 1999, and 886 °C.d in 2000) whole plant dry matter content (DMC) more or less doubled. The relation between DMC and T_{sum} could be described with the equation $DMC = 77 \times e^{0.0018 * Tsum}$ for Vitaro, and with $DMC = 76 \times e^{0.0018 * Tsum}$ for Volens ($R^2 = 0.95$; n = 18; in both cases). There was no statistical difference in whole plant DMC between the two cultivars (Figure 4B).

As Vitaro FW was higher than Volens FW, and DMC did not differ statistically between cultivars, Vitaro DW was also higher (Figure 4C); on average over all samples the difference was 30 g. The relation between DW and T_{sum} could be described with a linear function: $DW = 0.40\ T_{sum} - 68$ ($R^2 = 0.88$; n = 18) for Vitaro and $DW = 0.38\ T_{sum} - 85$ ($R^2 = 0.94$; n = 18) for Volens.

Whole plant dry weight increased markedly from anthesis onwards as a result of the development of the main ear. The dry weight of the other plant parts remained almost constant, which resulted in a considerable increase in the mass fraction of the

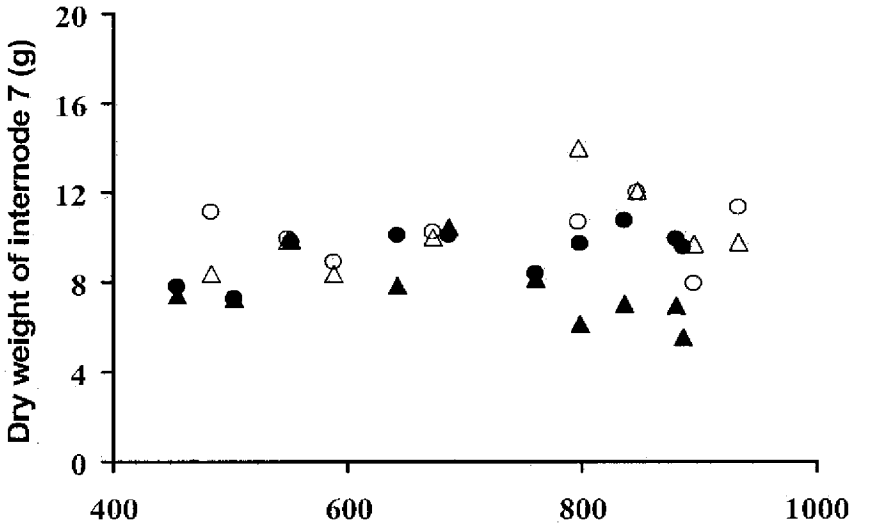

Figure 3. Relationship between dry weight of internode 7 and temperature sum (T-sum) for Vitaro and Volens maize plants. △ Vitaro 1999; ○ Volens 1999; ▲ Vitaro 2000; ● Volens 2000.

ear from anthesis onwards (Figure 5). The main ear was mostly positioned on internode 11, and in some cases on internode 10 or 12. The proportion ear dry weight : whole plant dry weight was similar for both cultivars throughout the growing period, and reached a maximum of 700 g kg^{-1}. Grain dry mass of the main ear reached a proportion of about 500 g per kg total DW for both cultivars (data not shown).

Fermentation characteristics of stem samples

The fermentation characteristics of whole stem samples are summarized in Table 4. Sampling date and cultivar had a statistically significant effect ($P < 0.001$) on the cumulative gas production after 72 hours (GP72) of incubation in buffered rumen fluid. Year had no statistically significant effect on GP72, but there was a statistically significant year × cultivar interaction ($P = 0.04$). Sampling date, cultivar and year had a statistically significant effect ($P < 0.001$) on gas production of cell wall material (a2). There was no significant year × cultivar interaction for this parameter ($P = 0.1$).

As for gas production of soluble components (i.e., cell content) (a1), there were statistically significant effects of year ($P < 0.001$), cultivar ($P = 0.02$), and sampling date ($P = 0.008$). But also the interactions year × cultivar ($P < 0.001$), cultivar × sampling date ($P = 0.004$), and year × cultivar × sampling date ($P < 0.001$) were statistically significant. For Vitaro, the a1 value and T&T value were surprisingly low at harvest date 9 October 2000.

Tilley & Terry digestibility (T&T) was significantly affected by cultivar and sampling date ($P < 0.001$ in both cases), but not by year ($P = 0.06$).

The time at which half of the maximum gas production of the non-soluble components (cell walls) was reached (b2) was slightly shorter for Vitaro than for Volens, and

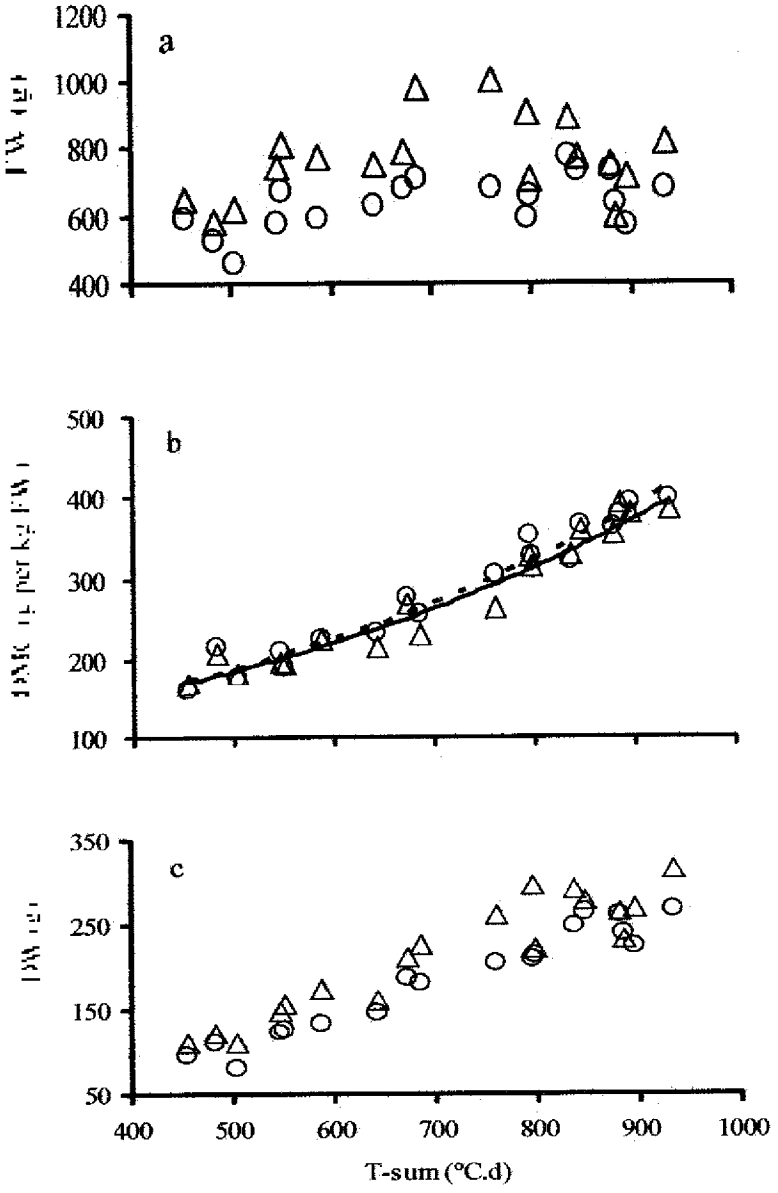

Figure 4. Relationship of fresh weight (FW; a), dry matter content (DMC; b) and dry weight (DW; c) temperature sum (T-sum) for whole plants of Vitaro and Volens maize sampled during the growing seasons of 1999 and 2000. (△ Vitaro, ○ Volens)

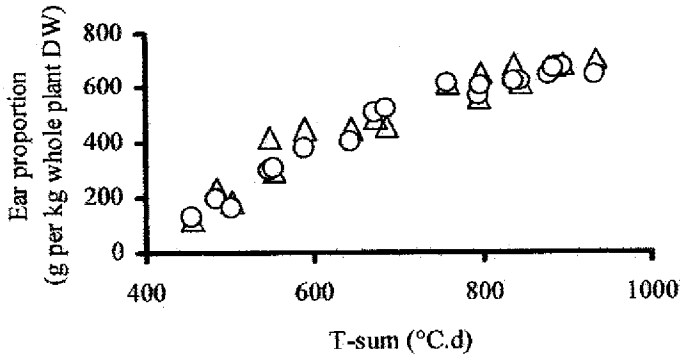

Figure 5. Relationship between proportion of dry weight in the ear and temperature sum (T-sum) of Vitaro and Volens maize plants sampled during the growing seasons of 1999 and 2000. (△ Vitaro, ○ Volens)

shorter for samples taken at anthesis than for samples taken at the end of the growing period (ensiling stage).

Figure 6 shows the effect of incubation period on the gas production rate of the

Table 4. Whole stem fermentation characteristics of Vitaro and Volens maize plants sampled at anthesis (August) or at the end of the growing season (ensilage stage) (September/October) and temperature sums (T_{sum}), in the years 1999 and 2000.

Fermentation characteristic[1]	Vitaro				Volens			
	1999		2000		1999		2000	
	3 Aug.	27 Sept.	3 Aug.	9 Oct.	3 Aug.	27 Sept.	3 Aug.	9 Oct.
T&T	79	70	74	61	68	57	63	61
GP72	308	287	303	261	281	251	270	270
a1	69	70	52	18	51	42	39	50
a2	134	121	147	137	123	109	132	114
b1	1.1	1.4	1.2	1.7	1.3	1.2	1.3	1.2
b2	9.9	11.0	10.2	11.4	10.6	13.5	10.3	11.4
T_{sum} (°C.d)	483	895	503	886	483	895	503	886

[1] T&T = Tilley & Terry digestibility (%); GP72 = gas production (ml per g organic matter) after 72 hours of incubation; a1 = GP3 (gas production after 3 hours of incubation); a2 = GP20–GP3; b1 = time (h) to reach 50% of a1; b2 = time (h) to reach 50% of a2.

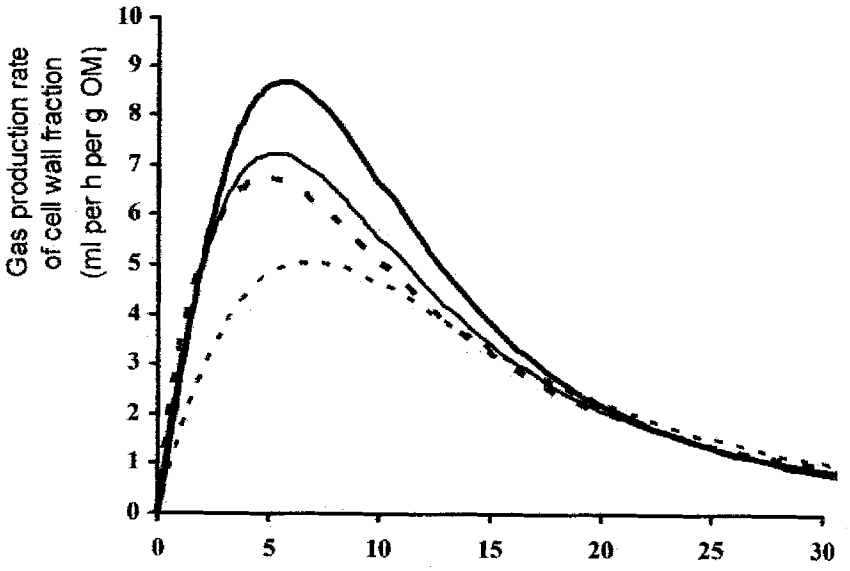

Figure 6. Gas production rate of insoluble components of Vitaro (thicker lines) and Volens (thinner lines) maize stem samples at anthesis (solid lines) and ensiling stage (interrupted lines) in the 1999 season.

insoluble components of the 1999 samples. Gas production rate differed notably between cultivars and between sampling dates. Vitaro samples taken at the ensiling stage had about the same gas production rate as Volens samples taken at anthesis. The difference in gas production rate between samples taken at anthesis or at the ensiling stage was smaller for Vitaro than for Volens. Gas production rate reached a maximum after 6 hours of incubation.

Discussion

Choice of internode and identification of the internode number were important aspects of sampling. As each leaf is accompanied by an internode, leaf counting was a straightforward method to identify internode number. The internode accompanying leaf 1 was designated as internode 1, whereas Morrison *et al.* (1994) designated this internode as internode 2. The first leaf is spatulate and could easily be identified by its form; all other leaves were acuminate. The first leaf was visible in the early stages of growth, but later on was lost or drawn into the soil. As growth proceeded, leaf senescence made it increasingly difficult to identify leaves and internodes by mere counting. To increase the reliability of the method, total leaf length was measured as soon as the leaf was fully-grown. Bos *et al.* (2000) found that within a particular cultivar leaf insertion number was well correlated with mean leaf length. Using mean leaf length, it was possible to identify leaf number and thus internode number easily and reliably.

In 2000, elongation of internode 7 of Vitaro and Volens was measured over a peri-

od of two weeks, but only started when elongation was already in progress. At the first recording, the length of internode 7 of Vitaro and Volens was 7.7 and 9.4 cm, respectively (Table 2). Eight days later, elongation appeared to be complete. For greenhouse-grown maize plants Morrison *et al.* (1994) found a period of internode elongation of 10 days.

In this study the dry weight of individual internodes remained constant up to a T_{sum} after anthesis of at least 200 °C.d, which is approximately one month (see Figure 3 for internode 7; data for other internodes not shown). Cell wall differentiation of internode 7 is thought to be complete at anthesis, which in the Netherlands usually occurs at the end of July or early in August. It can be assumed that cell wall degradability changes little after anthesis. Therefore, any difference in cell wall degradability between cultivars should already be present at anthesis. However, Cone & Engels (1993) observed a decrease in cell wall degradability after anthesis for the internode bearing the ear, which was accompanied by an increase in lignin content until the beginning of September. Morrison *et al.* (1994) observed that maize stems elongate in a sigmoidal pattern, which arises from the variable growth rates of the individual internodes. Although elongation of the stem is usually completed at anthesis, it seems likely that differentiation, i.e., secondary cell wall growth and lignin deposition, continues for some time after that, at least in the upper internodes. This was also observed in perennial ryegrass (Groot *et al.*, 2003). The mass of digestible cell wall in maize stems, however, may decline after anthesis (Deinum & Struik, 1991).

In a single internode, differences in rate and extent of fermentation can be traced back to differences in chemical composition, anatomical structure, and perhaps physical properties of the cell walls. It is unlikely that changes in cell wall chemical composition or anatomy occur after lignin deposition is complete. To investigate the cause of changes in cell wall fermentability, it is necessary to monitor anatomical structure, chemical composition and fermentation kinetics throughout the growing period.

In the comparison of the two forage maize cultivars, the same internode number was sampled, i.e., internode 7. As in Vitaro this was the second internode above the soil surface and in Volens the third, it might be questioned whether internode 7 fulfilled the same physiological function in both cultivars. Combined with the difference in internode length (Volens > Vitaro; Table 3) the different position above the soil surface resulted in a different height above the soil of 30 to 40 cm. However, the number of internodes per plant was the same and the main ear was positioned on the same internode in both cultivars. So it is likely that position and function of internode 7 within the plant were the same in both cultivars.

As yet, it is not clear which of the differences in internode- and stem morphology could account for differences in stem digestibility. Vitaro plants elongated slightly faster and developed a thicker stem with shorter internodes than Volens. Both, plants and individual internodes were shorter for Vitaro than for Volens (Table 3). The thicker and shorter internodes of Vitaro might affect stem strength positively, thus reducing lodging.

Whole plant digestibility as estimated with Near Infrared Reflectance Spectroscopy was 9% higher for Vitaro than for Volens (Advanta Seeds BV; unpublished results). With the present data, a comparison of quality characteristics was made to see whether

the difference in digestibility between the two cultivars could be attributed to stem characteristics. Ear weight amounted to 70% of total plant dry weight (Figure 5). Grain weight of the main ear was about 50% of total plant dry weight. The grains are known to be highly digestible.

A difference in whole plant digestibility between cultivars of 9% and assuming no difference in grain digestibility would mean a difference of 18% in digestibility for the rest of the plant. At the end of the season, the rest of the plant is made up of about 18% stem, 15% leaf and 17% husk / shank fractions, each of which consists of cell contents and cell wall material. From fermentation characteristics (Table 4) it was calculated that in 1999 the difference in stem cell wall fermentability (a2) between Vitaro and Volens was 8.9% at anthesis and in 2000 20.2% at the ensiling stage. It is likely that a similar difference in cell wall fermentability occurs in other cell walls. The data suggest that a substantial part of the differences in whole plant digestibility could be attributed to cell wall fermentability of the whole plant. Some reports (e.g. Deinum & Struik, 1989), however, also indicate a small decrease in digestibility of the grains and this decrease may also show some genetic variation.

The maximum amount of gas produced by fermentation of the soluble components (a1) (Table 4) was highly variable, with various interaction effects. This may be due to variable sugar contents of the cell content. Weather conditions and sampling time greatly influence sugar contents of the cells. In contrast, a2 did show statistically significant cultivar, year, and sampling date effects, but there were no significant interactions between these factors. Sampling date and cultivar effects were as expected. The year effect could be due to the large difference in weather between 1999 and 2000, the former being relatively dry and warm, and 2000 relatively cool and wet. This could have influenced cell wall formation, and therefore the fermentation characteristics of the cell walls.

Differences in stem cell wall fermentability are required to make maize cultivars suitable models for digestibility studies. From the data presented it can be concluded that the two forage maize cultivars studied are suitable as a model system to investigate differences in fermentation of cell walls in the rumen. Further systematic studies will be conducted on these model cultivars. Areas to be investigated will be anatomy and chemistry of the stem, as well as fermentation characteristics. For the study of the latter, the gas production technique is highly suitable. It provides the possibility to study the fermentation process throughout the period of incubation, and – by means of curve fitting – to calculate the parameters of cell wall fermentation specifically.

Such studies need to include differences in fermentability within a selected internode at a specific time, differences among internodes at this time, differences in a selected internode throughout the growing period and differences among isolated tissues from the selected internode.

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