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Climate, nitrogen and grass. 4. The influence of age on chemical composition and in vitro digestibility of maize (*Zea mays* L.) and tall fescue (*Festuca arundinacea* Schreb.)

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Summary

In a field experiment with maize it was found that in vitro digestibility of leaves decreased somewhat during undisturbed growth, whereas digestibility of stems decrased appreciably. This decrease in leaf digestibility was caused by a lower digestibility of later developed leaves and by a slight decrease in digestibility during aging of each individual leaf.

In a pot experiment with tall fescue grown at different temperatures it appeared that a higher temperature caused higher concentrations of cell-wall constituents and lower digestibility, whereas during aging the percentage of cell-wall constituents remained constant or even decreased, digestibility decreasing slightly.

These results suggested that in a constant climate the effect of age on digestibility would be small, and that the great effect of age on forage quality in spring is mainly due to rising temperature and stem formation.

Introduction

Age of grass is an important factor determining forage quality and therefore much research has been carried out on this subject. In Northwestern Europe, however, different results are usually obtained in spring, summer and autumn (Frankena, 1941) suggesting that aging is a rather complex factor. This is not illogical when it is considered that aging in spring is associated with formation of stem and inflorescence and at the same time with rising temperature and light intensity, whereas in summer and autumn the grasses are generally vegetative and temperature and light intensity constant or declining.

Therefore, many workers investigated in the first instance the changes in morphological composition with changing age, separating the material into leaf lamina, leaf sheath and stem (i.a. Fagan and Trefor Jones, 1924; Waite and Sastry, 1949; Terry and Tilley, 1964). In this way they were able to present a partial explanation for the changes in chemical composition and decrease in nutritive value of reproductive grass in spring with increased age.

However, because of the continuous formation of new and the decay of old leaves, the leaf samples of older grass will be composed of later developed leaves which development may have occurred at other light intensities and temperatures. Therefore more detailed information on the effect of age may be obtained, if the effect of climate on forage quality is better understood. Research of Deinum (1966, 1968) indicates that the chemical composition and nutritive value of grass is mainly determined by the climate in which it is grown: grass contains less non-structural and more structural carbohydrates at higher temperatures and lower light intensities.

In order to obtain more exact information about the principles it is necessary to study the effect of age per se by considering single organs and by keeping constant as many factors as possible. For this purpose two experiments were carried out, one with a maize crop in which each single leaf number was studied and one with tall fescue in temperature controlled greenhouses. This grass was prefered to perennial ryegrass, because it is coarser enabling easier separation into leaf lamina and leaf sheath. The results of both experiments are described in this publication.

Material and methods

Maize

Seeds of cv. 153 R × 374 B of CIV were sown in a sandy soil on 29 April 1964, at a density of 133,000 seeds/ha (row spacing 75 cm, plant spacing 10 cm). The experimental plot of 414 m^2 was fertilized with 42 kg P/ha in October 1963, and 46 kg N plus 100 kg K/ha in March 1964 and again with 24 kg P, 58 kg N and 27 kg Mg/ha on 29 April. Seed emergence occurred on 15 May, after which date plants were selected at random from the experimental plot on 12 dates with rather regular intervals until October 1964.

On the sampling dates the selected plants were quickly transported to the laboratory and analysed for length, fresh and dry weight of each individual leaf number, and dry weight of leaf sheath + stem, tassel and whole ear. Leaf 1 was the first developed leaf. After drying and grinding alle samples were analysed for the percentage of crude fibre (% cf) and some for crude protein (% cp) and ash (% ash). More recently in 1969, the samples were also analysed for in vitro true digestibility of organic matter (D_{vitro}), percentage of cell-wall constituents (% cwc), permanganate lignin, acid-detergent fibre (% adf) and SiO_2 according to the procedures of Van Soest (viz Deinum et al., 1968). In vitro digestibility of cell-wall constituents (D_{cwc}) could be calculated from these data.

Tall fescue

Thirty-one vegetative tillers of tall fescue were planted in 5-litre plastic pots filled with sandy soil on 14 February 1964, adequately fertilized with minerals and grown in a greenhouse at about 15° C, until 13 March. At that date the pots were distributed over three greenhouses at temperatures of 15/10, 20/15 and 25/20 day and night temperatures in °C and the tillers cut, marked with pigeon rings and allowed to regrow until 7 April, at which date the tillers were reclipped and the experiment commenced.

After this start five pots per temperature treatment were selected at random on each of the nine sampling dates between 29 April and 24 June. During sampling the grass was harvested in such a way that ringed (old) and non-ringed (young) tillers were counted and kept apart. In addition the old tillers were separated into leaf lamina and leaf sheath. All fractions were dried, weighed, ground and analysed for crude-fibre content. More recently in 1969, these samples were also analysed for the same constituents as the maize plants.

Results

Maize

Weather conditions were rather favourable for maize production in 1964 and soil water availability was adequate during the whole season in this rather moist soil. The development of the maize plants and the in vitro digestibility of the different morphological constituents during the season are shown in Fig. 1 together with light intensity and temperature.

The growth curves show the normal pattern except for two rather high points for stem weight which may be partly due to sampling errors. However some decrease in stem weight may be expected at grain filling stage, according to the findings of Meyers et al. (1939) and Becker and de Haan (1955). The in vitro digestibility lines for the whole crop and its constituents reveal a constant decrease with increased age as expected, suggesting that the results to be mentioned may be of general value. Contrary to the leaves the decrease in digestibility of the stem fraction was rather sharp and closely related to the concentration of cell-wall constituents and lignin.

Because of the separation procedure of the leaves it was possible to follow the life cycle of each leaf separately. However, for convenience the results of the 19 leaves, grouped as five fractions of four leaves, are shown in Table 1. This table reveals that with increasing leaf number dry weight increases up to the fourth group (leaf number 13 was the heaviest), but that in vitro digestibility of organic matter and of cell-wall constituents decreases. % cwc, % cf and % lignin are enhanced, although the latter not significantly. This indicates that with advancement of the season leaves are formed with higher concentrations of cell-wall constituents and with a lower digestibility. However these data are averages and age is not involved.

According to the results of Deinum (1966, 1968), it is suggested that these decreasing digestibilities may be caused by the increasing temperatures from spring into summer; the higher leaf numbers are formed at higher temperatures. This suggestion is supported by the results of another experiment which showed that the same leaf numbers had higher % cell-wall constituents and lower digestibilities at a later sowing date (Dirven, 1966, unpublished data).

The periodic sampling technique enabled the changes in each leaf number to be followed from emergence through full expanding until decay and it was found that all the leaves behaved in a similar way. Therefore the complete behaviour of one leaf can be demonstrated and leaf number 13 was selected for this, because it showed the most characteristic points, closest to the sampling dates (Fig. 2).

From this figure it may be seen that during leaf development adult leaf weight is achieved about two weeks after the adult length is attained. The percentage of cell-wall constituents and crude-fibre were highest at the moment of adult leaf length, followed by a decrease until adult leaf weight was attained. After that the percentages remained constant.

Calculation of the quantity of cell-wall constituents and crude fibre of the leaf revealed that the maximum amount was already present at the moment of adult leaf length, indicating that cell-wall development is completed during the period of leaf elongation. Any further change in % cwc and % cf after leaf elongation is therefore associated with changes in influx and efflux and cellular contents. Consequently, no symptoms of aging occur in the leaf of maize as far as % cwc and % cf are concerned.

Nevertheless, when in vitro digestibility of organic matter and of cell-wall constituents

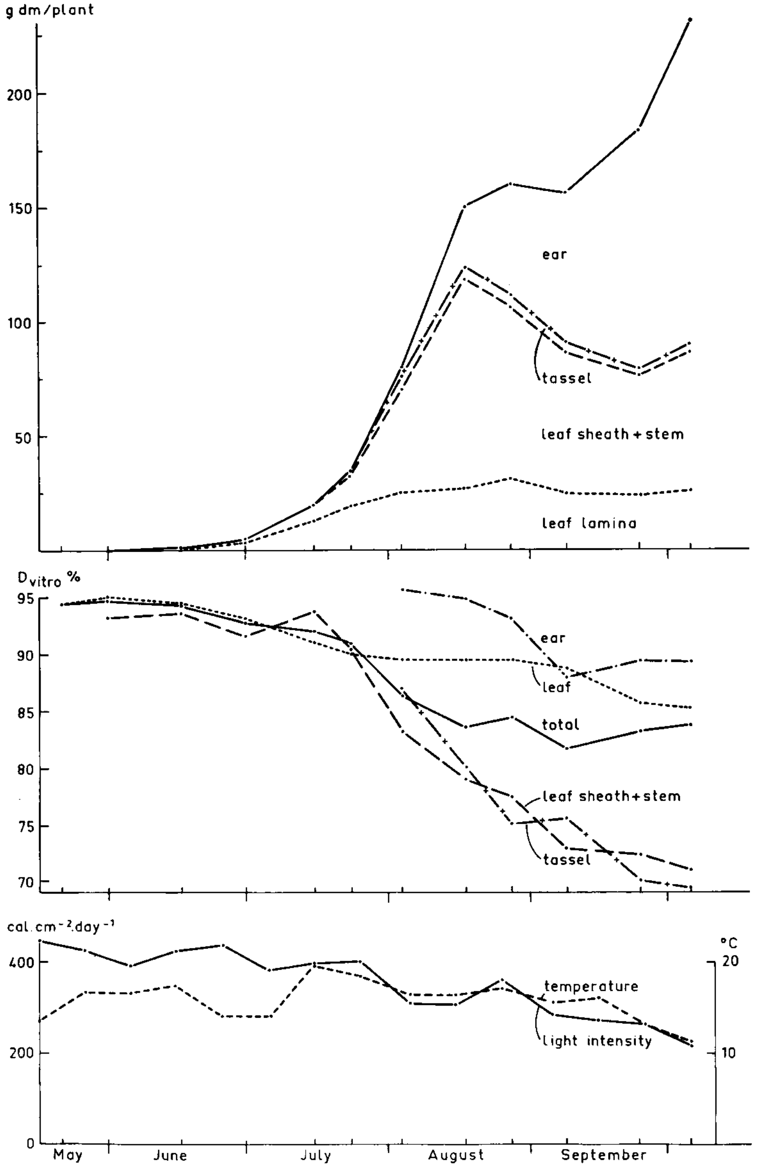

Fig. 1. The trend in dry weight and in vitro digestibility of different fractions of maize during the growing period, together with the average light intensity and temperature per decade.

is concerned, definite aging effects do occur, in that digestibility decreases with age. This decreasing digestibility of cell-wall constituents seems not to be related to the % of lignin, for the concentration of this constituent remained almost constant. However, Mühling (1963) and Crössmann (1967) did find an increase in % lignin, although they

Table 1. Characteristics of different groups of leaf number in maize.

Leaf number	Date of first presence	Leaf weight	D_{vitro}	D_{cwc}	% cwc	% cf	% lignin	% SiO_2
1– 4	21 May and 1 June	0.05	94.2	88.2	42.6	16.0	—	—
5– 8	16 June	0.56	92.2	83.4	45.5	18.7	2.15	4.82
9–12	30 June	2.24	89.4	80.8	55.0	22.4	2.60	2.73
13–16	15 July	2.48	88.4	79.9	57.8	23.5	2.73	2.35
17–19	3 Aug.	0.97	88.6	80.8	59.0	23.4	2.52	3.30

used an other procedure of lignin analysis and found, against expectation, higher concentrations in leaves than in stems. This decreasing digestbility seems to be associated with % SiO_2. This agrees with the findings of Jones and Handreck (1969) who suggest that silica present in the soil water enters the plant as a mass flow. The water is transpired by the leaf, whereas silica remains inside as a deposit in the cell walls, where it may

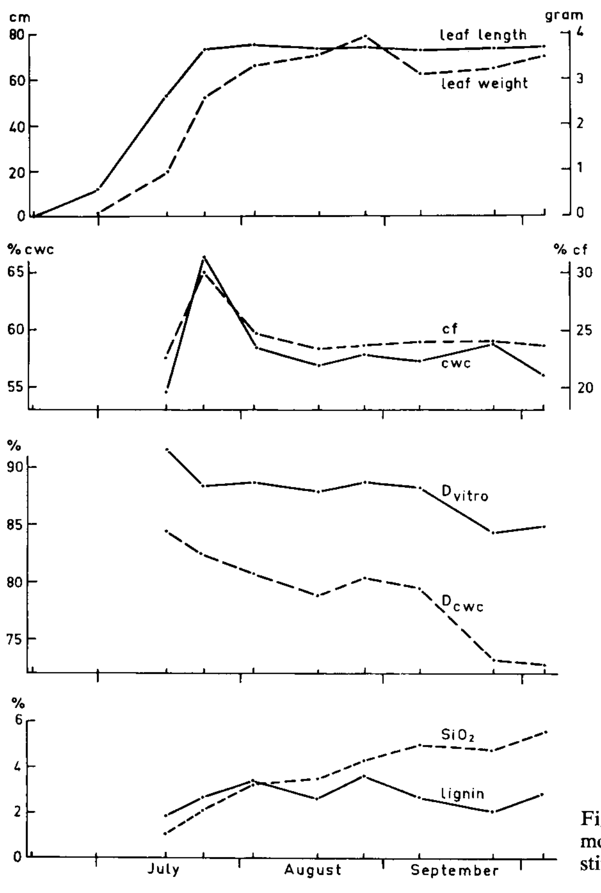

Fig. 2. The effect of age on some morphological data and chemical constituents of the thirteenth leaf of maize.

inhibit cell-wall digestion in a similar way as lignin (Van Soest and Jones, 1968). However, some doubts as to this effect of silica on digestibility have recently been expressed (Hartley et al., 1970).

Summarizing this experiment it may be concluded that the initially rather sharp decrease in digestibility during growth and development of maize is mainly due to the relative increase in the stem fraction and its decreasing digestibility. In addition some decrease occurs in the leaf fraction which is caused both by a lower digestibility of later developed leaves and by a similar decrease in digestibility of the leaves during aging. However, digestibility of the whole crop increases again during the grain filling period due to the increasing proportion of the highly digestible ear.

Tall fescue

The highest production in this experiment was achieved at 20/15° C and the lowest at 15/10° C. However, the highest tiller number was found at 15/10° C in agreement with the findings of many authors that the optimum temperature for tillering is 5-10° C below the optimum temperature for shoot growth (i.a. del Pozo, 1963). During the experiment a decrease in the number of old living tillers was found, especially at 25/20° C; only half the number of old tillers was still present by the last harvest at this temperature.

The information on average tiller weight and number is summarized in Table 2 which also demonstrates the effect of temperature on chemical composition and digestibility. It shows that % cwc, % cf and % lignin are enhanced by higher temperature, whereas D_{vitro} and D_{cwc} are decreased. However these effects were smaller in the range between 20/15° C and 25/20° C than between 15/10° C and 20/15° C, suggesting that the temperature effect is smaller above the optimum temperature than below. These results agree very well with other findings (Deinum, 1966; Deinum and Dirven, 1972).

Table 2 indicates also that the average digestibility of old and young tillers is almost the same, however, there are some differences in digestibility between leaf lamina and leaf sheath. At all temperatures except 25/20° C, %cwc, % cf and % lignin of the leaf sheath are lower than those of the leaf lamina which may be due to accumulation of

Table 2. Effect of temperature on production and chemical composition (in % of dm) of different fractions of tall fescue (average of 9 harvests)

	Number of tillers	Average dry weight per 100 tillers (g)	D_{vitro}	D_{cwc}	% cwc	% cf	% lignin
15/10° C							
old tiller leaf	28	25.1	94.7	87.0	40.7	20.7	2.01
old tiller sheath		11.3	92.8	80.3	38.0	18.9	1.79
young tillers	120	11.9	94.3	85.5	40.2	20.0	1.99
20/15° C							
old tiller leaf	25	39.2	89.7	77.7	46.6	24.0	2.45
old tiller sheath		18.0	87.1	70.2	44.7	22.4	2.47
young tillers	83	24.7	89.6	78.0	47.1	23.3	2.40
25/20° C							
old tiller leaf	18	39.6	88.7	76.8	48.6	25.4	2.63
old tiller sheath		20.1	83.5	66.6	49.6	23.8	2.71
young tillers	72	28.8	87.8	75.5	49.9	25.0	2.80

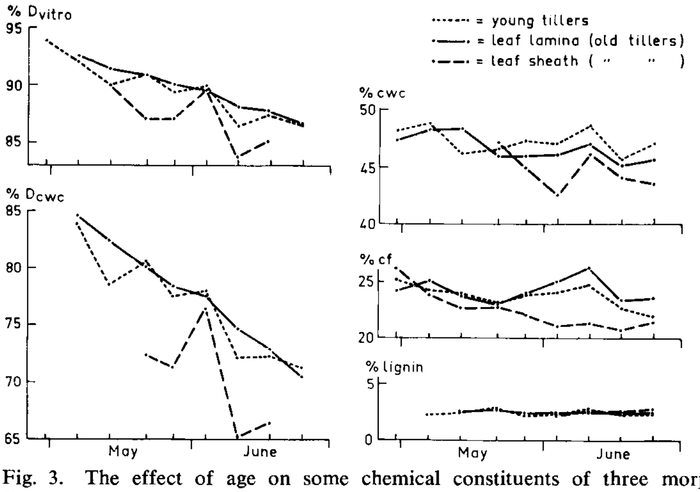

Fig. 3. The effect of age on some chemical constituents of three morphological fractions of tall fescue at a temperature of 20° C during the day and 15° C at night.

reserve carbohydrates in the leaf sheath. Nevertheless, D_{vitro} and D_{cwc} of the leaf sheath are lower than of the leaf lamina, although lignification seems not to be cause of this difference.

Up to now this experiment has revealed results largely in agreement with what was expected. However, examination of the effect of age on chemical composition showed that under these conditions of constant temperature $^0/_0$ cwc, $^0/_0$ cf and $^0/_0$ lignin did not increase with age: $^0/_0$ cwc and $^0/_0$ cf even tended to decrease at 15/10° C and 20/15° C (viz. Fig. 3 in which for convenience only the results of 20/15° C are presented). This was true for leaf lamina and leaf sheath of old tillers as well as for the young tillers. Maybe this decrease is associated with increased light intensity.

However D_{vitro} and D_{cwc} decreased appreciably with age which cannot be ascribed to lignification. This suggests that as far as the chemical constituents analysed are concerned no age effects are found, but these effects are definite in the digestibility of the leaves of tall fescue.

Finally, it may be mentioned that the effect of age on D_{vitro} of leaves of tall fescue is of about the same order as on that of maize leaves.

Discussion

However different in set up and execution, the results of both experiments show a great deal of agreement. For instance $^0/_0$ cwc and $^0/_0$ cf remained almost constant in the leaves of maize and tall fescue during aging, whereas D_{vitro} and D_{cwc} decreased. The average concentrations of these constituents and digestibilities were almost equal in both experiments as well, although maize leaves showed a higher $^0/_0$ cwc than tall fescue leaves, nor was there much difference between the lignin concentrations. However, in these experiments maize contained much more SiO_2 than tall fescue which was almost free from this substance.

In the experiment with tall fescue it was not possible to separate the leaves into specific numbers like in maize. Therefore all the leaves of the old tillers were permanently combined during separation. The total number of living leaves on each tiller was generally four in agreement with Alberda's findings (1966) in *Lolium perenne*. This implies that at the end of the experiment other leaves are investigated than at the beginning. These later developed leaves were larger than the earlier in accordance with the general findings that leaf size increases with leaf number in vegetative tillers.

All tall fescue leaves were formed at a constant temperature within a temperature treatment which may indicate that the difference between early and late leaves is rather small as far as % cwc is concerned. However, the difference in digestibility is greater.

The maize leaves were formed at different temperatures, but if they had been formed at the same temperature they might have shown almost constant concentrations of cell-wall constituents and crude fibre, with possibly somewhat lower concentrations in the top leaves, because of higher light intensities. The results of Ehara's experiment (1959) support this supposition, although his data refer only to the silk stage.

Both Tables 1 and 2 reveal that leaves contain a higher concentration of cell-wall constituents and show a lower digestibility at a higher temperature. Consequently the results of both experiments may indicate that the same will happen with the rising temperatures in spring. Stem formation will cause an additional decrease in digestibility.

These combined effects of age, stem formation and climate may also be inferred from the literature, for in many cases it was found in spring that on the same sampling date late varieties of grass species had a higher digestibility than early varieties due to a smaller stem fraction. However at the same stage of development the reserve is found, because the late varieties attain this stage at a later date at a higher temperature (i.a. Mowat et al., 1966).

In contrast, the sharp drop in temperature in autumn may cause a lowering of % cwc which was indeed found in experiments carried out in autumn in the Netherlands (Deinum, unpublished data, 1970) and in the United States of America (Deinum, 1969). This may counteract the decreasing digestibility of cell-wall constituents and even cause an increase in dry-matter digestibility with increasing age.

Finally, it should be noticed that in both experiments crude fibre was not a reliable reference for estimating digestibility. In maize it suggested a constant digestibility of leaves during aging after attaining adult leaf weight, whereas in tall fescue it even assumed an improvement in digestibility with age. In addition % cwc and % lignin neither were good indicators. Fortunately, D_{vitro} determinations prevented these erroneous conclusions.

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