Effect of Nitrogen Supply on Early Growth Development and Nitrate Reductase Activity in Two Cassava Cultivars

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Abstract

Leaf growth, development and nitrate reductase activity (NRA) of two cassava cultivars in relation to N-supply are discussed. Differences in NRA between the cultivars and variations in enzyme activity with leaf and plant ontogeny were found. Significant decreases in leaf NRA occurred at the time of tuber initiation and was coincident with reduction in the rate of shoot growth. High N-supply stimulated leaf growth and NRA was shown to be correlated with leaf growth rate and final leaf size. A regulatory role for NRA in assimilate distribution is suggested.

Introduction

Shoot and leaf growth were identified as important physiological factors affecting yield in cassava (Doku, 1965, Holmes and Wilson, 1976). Nitrogen applications, either as fertilizer or nutrient solution have been shown to increase shoot growth in all root crops (Watson, 1963; Walter, 1966; Chapman, 1967; Dyson and Watson, 1971; DasGupta and Ghosh, 1973) as well as cassava (Krochmal and Samuels, 1967; Fox *et al*, 1979). High nitrogen applications in potato increased plant leaf areas, rates of leaf production and reduced leaf durations (Watson, 1963).

Brouwer (1962) noted that increased nitrogen supply increased plant dry weight and shoot to root ratios, while a reduction in nitrogen restricted the utilization of carbohydrate supply to the root and tuber. Therefore, nitrogen makes the shoot sink a stronger competitor for the available carbohydrate.

Nitrate reduction to nitrite by the adaptive enzyme nitrate reductase is the first sequence of metabolic events leading to protein synthesis. It has been demonstrated that this enzyme could become a limiting factor for crop growth development and yield, particularly in cereals (Beevers and Hageman, 1969). Rapid shoot growth in sweet potato in response to high nitrate supply was also found to be associated with high nitrate reductase levels in the leaf (Wilson and Knox, 1975). Therefore, Wilson (1975) considered that the pre-eminence of shoot growth under conditions of high nitrogen supply might be due to the capacity of this enzyme to direct carbohydrate to protein synthesis in the leaf rather than to storage in the root.

Nitrate reductase activity is known to vary with species as well as with plant and leaf age. Highest activities are found in young plants and decrease with leaf and plant age recorded (Wallace and Pate, 1965, 1967; Harper and Hageman, 1972). Wilson (1974, 1975) suggested that reduction in leaf nitrate reductase activity after tuber initiation might be advantageous for yield development in root crops. Therefore investigations of nitrate reductase levels in cassava during plant ontogeny were conducted to investigate whether variation in activity occurred.

In this paper, nitrogen supply was used to manipulate shoot growth in two cassava cultivars with contrasting patterns of dry matter distribution. Lamina nitrate reductase activity was measured in relation to such nitrogen supply as well as parameters of shoot and leaf growth. The study was conducted in two experiments which are described separately.

Materials and Methods

Experiment I

Plant Culture and Treatments. Two cassava cultivars, Maracas Blackstick (MBS) and Whitestick 2 (WS2) were cultivated in pots in two plantings from 25th March to 18th October 1976 and 18th November 1976 to 7th June 1977. These are referred as the 1976 and 1977 crops respectively.

The 1976 and 1977 experiments included 200 and 264 tins (23 x 23 x 35 cm), filled with 0.036 m^2 of a 1:2 soil sand mixture, arranged with 0.7 m between tin centers in the row and 1.2 m between rows, respectively. In 1976, there were 10 rows of 20 tins each, while in 1977 there were 12 rows containing 22 tins each. The outer rows acted as guard rows in 1977.

In the 1976 crop, 100 similar planting sticks for each cultivar (ave. wt. 37.6 g and 39.4 g for MBS and WS2 respectively) and in 1977 132 setts per cultivar were chosen (17.1 g for MBS and 19.9 g for WS2). Setts of both cultivars were randomly selected and planted horizontally, one to a tin, just below the soil surface. The experiment was established as a fully randomized design.

Following germination, all shoots were removed except one and this single shoot condition was maintained throughout the experiment.

In both crops, after 4 weeks of growth, 80 plants in each cultivar were selected with similar node numbers and half of these were treated with 100 ml of nutrient culture solution containing 210 ppm nitrogen and designated N2. The remaining 40 plants per cultivar received 100 ml solution containing 21 ppm nitrogen (N1). These applications were repeated at weekly intervals.

In 1976, the smallest leaves visible on each treated plant were identified and tagged after 36 days (T1) and 91 days (T2) after planting respectively. In 1977, leaves were also identified but at 63 days (T1) and 112 days (T2) after planting.

Sampling. Total leaf production (node numbers) were recorded at intervals throughout both experiments.

At intervals during the development of identified leaves, one leaf per plant was harvested from plants of each treatment, chosen at random. The number of plants varied between 3 to 8 depending on leaf size. These leaves were labelled and placed in plastic bags which were immersed in ice and taken to the laboratory. All samples were taken

between 9:00-9:15 a.m.

Lamina fresh weights were recorded as were petiole length, midlobe lengths and widths and lobe numbers for each leaf. Lamina areas were calculated from the leaf rectangular area by the method of Spencer (1962).

Nitrate reductase activity. Each sample of leaves was assayed for *in vivo* nitrate reductase activity (NRA), by a modification of methods of Mulder *et al* (1959) and Klepper *et al* (1971). Circumstances beyond the author's control prevented the assay of samples taken 14 days after identification in leaves appearing 63 days after planting, in the 1977 crop.

The following assay procedure was adopted for routine *in vivo* determinations of NRA in cassava leaves. Freshly collected laminae were sliced into 0.5 to 1.0 cm^2 fragments, washed twice in distilled water and dried between filter papers before being weighed into 0.2 g samples. These were placed in 25 ml sample tubes with 4.5 ml 0.1M phosphate buffer (pH 8.8) containing 5% propanol. Tissue fragments were then infiltrated twice, under vaccuum, until all surfaces were visibly wetted. Then 0.5 ml of 1M potassium nitrate were added to all samples, the mixture placed in the dark at room temperature for one hour and shaken at regular intervals. After this time, 2 ml of nitrite reagent containing a 1:1 v:v mixture of 1% sulphanilamide in 3 N hydrochloric acid and 0.02% N-1 napthylethylene diamine dihydrochloride. After 15 minutes the optical density of the samples was determined on a Unicam S.P. 600 Spectrophotometer at 540 nm and the nitrite concentrations obtained from previously prepared standard curves. NRA was expressed in terms of $nMNO_2/g(f.\ wt.)^{-1}.h^{-1}$.

Experiment II

Plant culture and treatments. Forty-five similar planting sticks (8 cm long) of MBS and WS2 were prepared, as previously described, and planted in tins. These tins were arranged in six rows of 15 tins per row, there being 60 cm between tin centers within the row and 120 cm between rows.

The experiment was established as a randomized split block design, with 3 replicates. There were 15 plants of each cultivar per replicate randomly divided into 5-harvest blocks, which were sampled at regular intervals between 29 and 99 days after planting. One harvest block was also used for NRA estimations from a single leaf, at intervals between 21 and 85 days after planting.

A complete culture solution containing 210 ppm N was applied at weekly intervals at a rate of 100 ml per plant. All other cultural treatments were as described in Experiment I.

Sampling. Numbers of leaves produced were recorded for each plant.

Nitrate reductase activity. The leaf arising from the 7th node, counted basipetally from the plant apex, was removed from three plants per replicate in each cultivar, from 21 days after planting. Data was recorded from this leaf as previously described *in vivo* NRA.

For canopy NRA estimations, samples were taken from each replicate at 29, 43, 57, 78 and 99 days after planting. All leaf blades were removed from 3 plants per cultivar in each replicate, bulked and placed in plastic bags immersed in ice. Following weighing, these samples were assayed for NRA as previously described and NRA expressed either on a gm. F.Wt. basis or as activity per leaf or as total canopy activity. Remaining leaf tissue was reweighed and dried at 80°C with the rest of each plant and the plant organ dry weights recorded.

Results

Experiment I

Total leaf production. Total leaf production per plant during ontogeny in both crops, averaged 97.1 at 188 days after planting in 1976 and 82.2 after 201 days in 1977 (Fig. 1). These were achieved at average rates of 0.48 n/p/d[1] in 1976 and 1977 (Table 1). Leaf production was approximately constant with time in both crops, particularly in 1977 (r – 0.977). However, there was a decrease in the number of nodes produced per day with plant age. In 1976 mean rates of leaf production were 0.63 n/p/d between 27 and 63 days after planting and 0.38 n/p/d between 63 and 167 days.

Nitrogen applications increased total leaf production at final harvest in both crops (Fig. 1) being highly significant in 1976. There was a higher rate of leaf production in the N2 plants (0.52 n/p/d) than in N1 plants (0.45 n/p/d) in 1976. Differences in the rate of leaf production between treatments were also evident in 1977 (Table 1).

Although the effect of nitrogen on total leaf production was similar in both cultivars, WS2 produced significantly greater node numbers than MBS in both crops (Fig. 1) due to higher rate of leaf production.

Leaf size. Final leaf sizes differed between experiments with petiole lengths (Fig. 2) and midlobe lengths (Fig. 3) being 17% longer and lamina area (Table 2) 54% greater in 1976 than 1977. In 1976, maximum petiole lengths decreased by 32% in leaves appearing at 91 days (17.0 cm) compared with those appearing at 36 days after planting (Fig. 2). Midlobe lengths (Fig. 3) and lamina area (Table 2) also decreased in the same period, by 15.2% and 32.0% respectively. Whereas in 1977, leaves appearing at 112 days had 15% longer petioles and midlobes as well as 43.6% larger laminae areas than leaves appearing at 63 days after planting.

N2 plants did not have significantly longer final petiole (Fig. 2) and midlobe (Fig. 3) lengths than N1 plants in leaves appearing at 36 and 63 days after planting, in 1976 and 1977 respectively. Leaves appearing at 91 and 112 days after planting in 1976 and 1977, respectively had longer petiole lengths in N2 plants than N1. These differences were significant throughout development in 1976 and at 20 and 34 days after leaf appearance, in 1977. Midlobe length (Fig. 3) and lamina area (Table 2) were also greater in N2 than N1 plants.

There were no significant differences in average petiole and midlobe lengths between cultivars, irrespective of crop or plant age at leaf appearance. However, MBS had 8.6% and 12.6% larger lamina area than WS2, in leaves appearing at 36 and 91 days after planting in 1976. In 1977, MBS also had larger leaves.

Lamina fresh weight. Average maximum lamina fresh weight differed between 1976 (2.14 g) and 1977 (1.52 g) by 28.6% (Table 3). In 1976, leaves appearing at 36 days after planting weighed 46% more than those appearing after 91 days.

N2 treatments increased lamina fresh weight in all mature leaves by 13.5 to 39.5% compared to N1 plants, however these differences were not significant (Table 3). Maracas Blackstick had heavier leaves than WS2 in both years except in leaves appearing at 91 days after planting in 1976. However these differences were only significant in leaves appearing at 63 days after planting in 1977.

Lamina nitrate reductase activity. Plant nitrate reductase activity (NRA) was affected by cultivar, plant age and season in cassava plants grown in 1977 and 1976. (Fig. 4). Nitrate reductase activity levels varied considerably with leaf ontogeny in all leaves. There were rapid increases in average enzyme activity with leaf age to a maximum

between 8 and 12 days after leaf appearance. Following this, NRA decreased to lower and relatively constant levels.

In both crops, maximum NRA declined with increasing plant age at leaf appearance. This effect was seen when experimental data from both crops were combined (Table 4). The reduction in activity was greater between leaves formed 36 and 91 days after planting in 1976 (94.9%) than in leaves appearing in 1977 (3.3%).

N2 plants had significantly higher (P = 0.05) maximum NRA levels in leaves appearing at 36 and 91 days after planting in 1976 and 112 days in 1977 (Fig. 4).

Differences in NRA were evident between the two cultivars, with MBS having significantly higher activities than WS2 throughout palnt and leaf development in 1977. In 1977, MBS also had higher NRA than WS2, but these differences were not significant.

Maximum lamina NRA coincided with the period of highest leaf growth rates, i.e. between 33 and 60% final leaf size. There was a weak association in leaves appearing 36 days after planting between rates of petiole length ($r = 0.47^{*}$) and midlobe length ($r = 0.61^{**}$) development during leaf extension growth (6-19 days after leaf appearance) and NRA in the same period. In the other leaves there were insufficient data points for analysis. However, it was concluded that increased NRA during early leaf growth resulted in faster leaf growth rates and final leaf sizes.

Relationships between maximum NRA levels and leaf size and weight were tested for plants in 1976 and 1977 (Table 5). There were positive and significant regressions between maximum NRA and final leaf size and lamina fresh weight respectively, in 1976. However, there were no correlations in 1977, where there were smaller treatment and age differences in NRA. So high plant NRA activities could lead both to rapid shoot and leaf development.

Experiment II

Dry matter production and distribution. Total dry weights per plant increased in both cultivars throughout the experiment (Fig. 5) and were 40.7% greater in WS2 (38 g) than MBS (27 g) at 99 days after planting. Patterns of dry matter distribution varied with plant age (Fig. 6), with the proportion of dry matter in the planting stick being reduced from an average 74% at 29 days to 21.6% after 99 days. During the same period, stem and lamina percentage dry weights increased from 4.9% to 22.7% and 11.6% to 33.9% respectively. Tuber development accounted for an increasing proportion of the total dry matter from 57 days after planting, rising to 13% after 99 days of growth (Fig. 7).

Since tubers were first identified at 57 days after planting, it was assumed that tuber initiation and bulking commenced in the period 43 to 57 days after planting. Tuber growth was greater in WS2 than MBS, with tubers of the former weighing 7.1 g (dm) or representing 18.5% of the total dry weight and in the latter, only 2.1 g (dm) or 7.4%, at 99 days after planting. Thus indicating a slower tuber growth rate in MBS in this experiment.

Total leaf production. Total leaf production data, from both "individual leaf and canopy sampling" dates are shown in Fig. 7. The two sets of data were similar, there being no significant differences in average rates of leaf production (0.38 nodes per plant per day for both individual and canopy sampling dates).

Alfhough leaf production was approximately constant during plant ontogeny, there was a slight reduction in the rate of leaf production, in both cultivars, with increasing plant age. Leaves were produced at an average rate of 0.41 per day during the period 20 to 55 days after planting and 0.34 per day from 55 to 99 days.

Nitrate reductase activity. "Individual leaf and canopy" NRA varied with plant age (Figs. 8 and 9). The average NRA of the leaf arising from the 7th node counted basipetally from the plant apex, rose sharply to a maximum of 7.94 $nMNO_2^-/g(f.\ wt.)^{-1}.h^{-1}$ and 6.18 $nMNO_2^-./leaf^{-1}/h^{-1}$ at 35 days after planting. Enzyme activity then dropped by 95.4% to an average of 0.31 $nMNO_2^-/g(f.\ wt.)^{-1}.h^{-1}$ at 55 days after planting.

The patterns of individual leaf NRA per unit lamina fresh weight and per leaf were similar as the average individual leaf fresh weights were constant (0.95 q) after initial increases in the first two harvests.

Maracas Blackstick had 50.8% greater NRA levels than WS2 at 35 days after planting. NRA in both cultivars declined with increasing plant age but MBS maintained significantly higher enzyme activities than WS2 (Fig. 8).

Average canopy NRA per unit leaf fresh weight was maximal $4.4nMNO_2^-/g(f.\ wt.)^{-1}.h^{-1}$ at 29 days after planting, and then declined during plant ontogeny to $0.094nMNO_2^-/g(f.\ wt.)^{-1}.h^{-1}$ after 99 days (Fig. 9). Therefore it was concluded that canopy NRA per unit leaf fresh weight declined during plant ontogeny.

Total NRA for the canopy was calculated for each harvest and the data shown in Fig. 9. Average NRA per shoot declined by 78% from 29 to 43 days after planting, before increasing at 57 and 78 days after planting, due to a combination of increased enzyme activities and plant leaf fresh weight. However, there was a 79% reduction in the total canopy NRA at final harvest from the initial enzyme activity. Thus indicating a general reduction in canopy NRA with increasing plant age.

Discussion

Differential levels of nitrogen applications affected shoot growth, leaf development and NRA in two cassava cultivars with different growth patterns. It was found that N2 plants had significantly increased leaf production when compared to N1 plants, although not until 60 days after planting in 1976 and 200 days in 1977. Percentage leaf fall was also increased by high nitrogen applications in both years, although not significantly. Therefore the number of leaves retained in each cultivar was unaffected by differential nitrogen applications until the later stages of plant ontogeny. In potato, nitrogen was shown to increase leaf production and retention, while rates of loss remained constant (Watson, 1963, Dyson and Watson, 1971).

Differential nitrogen applications did not affect individual leaf growth until 90 days after planting in 1976 and 112 days after planting in 1977. In these leaves, N2 concentrations stimulated petiole and lamina growth rates and so increased final leaf size.

The lack of a significant response to nitrogen until after the second month of growth in 1976 was considered to be due to sufficiency of soil N and/or planting sett nutrients for initial growth. A similar lack of response to applied nutrients until 2 months after planting was also shown in cassava by Hunt (1975), who ascribed the phenomenon to the mobilization of nutrient reserves from the planting material. Orioli *et al* (1967) also measured a slow uptake of nutrient from the soil during the first two months of cassava growth.

There were variations in rates of leaf production and also maximum leaf sizes during plant ontogeny. In 1976 and also in the brief 1977 experiment there were slight but definite reductions in rates of leaf production in older plants. As tubers were first identified between 43 and 55 days in 1977, it was assumed that tuber bulking

commenced in that period. Root investigations in 1976 also determined that tuber growth commenced prior to 60 days after planting. Therefore it was concluded that tuberization might be responsible for the reduction in leaf production.

Maximum leaf sizes and weights were reduced in leaves appearing between 36 and 91 days after planting in 1976, while in 1977, final leaf sizes and weights were similar in leaves appearing at 63 and 112 days after planting i.e. after the onset of tuber bulking. Leaf size changes were also shown in Colombian cultivars, where leaves increased to a maximum size and then decreased with plant age (CIAT 1974).

Fick *et al* (1973) established in sugar beet that there were relationships between root and shoot sinks based on specific priorities for assimilate distribution. Whereas, cassava cultivars exhibited continuous partitioning of dry matter into root and shoot sinks following tuber initiation (CIAT 1974, 1975), Loomis and Rapoport (1976) reported a change of foliage to root dominated growth in sugar beet. This latter mechanism appeared to be the case in the cassava cultivars grown in pots, where the number of branches were few.

In the pot trials here described, with plants having only 1-3 branches, tuber development apparently resulted in a change in the assimilate distribution pattern which reduced the photosynthate available for leaf production at each meristem and hence the rate of leaf production. Therefore, during early plant development the shoot apex and new leaf growth received the major proportion of photosynthate and so produced larger leaves at a faster rate than later in plant ontogeny, when the tuber sink developed and competed successfully for the available assimilate.

Leaf NRA was recorded in all crops and maximum activities ranged from 0.43 to 5.77 $nMNO_2^-$. g(f. wt.)$^{-1}$.h^{-1} in 1976 and 1977 respectively and 9.56 $nMNO_2^-$. g(f. wt.)$^{-1}$.h^{-1} in Experiment II. These values compared favourably with recorded NRA levels in other plant species. Klepper *et al* (1971) compared *in vivo* NRA levels in other plants species and obtained values ranging from 0.9 to 10.2 $nMNO_2^-$. g(f. wt.)$^{-1}$.h^{-1}.

Leaf NRA of many crops have been found to vary with leaf and plant ontogeny. NRA per gram fresh weight increased to a maximum at 50% final leaf size, which corresponded to a position at leaf node 7, and then decreased to low and constant levels. Similar reductions in NRA with increasing leaf age have been shown in cereals (Schrader *et al* 1974) and legumes (Harper and Hageman 1972). In these cassava cultivars, attainment of maximum leaf growth rates coincided with maximum leaf NRA, at which time there would be high demands for reduced N for amino acid and protein synthesis (Hedley and Stoddart, 1972; Maksymowych, 1973).

Nitrate reduction is the rate limiting step in the assimilation of nitrate into protein and has been related to shoot and leaf development rates (Hageman and Flesher, 1960). In the cassava leaves studied NRA was correlated to leaf development rates and also final leaf size and weight, with higher enzyme activities resulting in larger leaves. Therefore lamina NRA could be an important determinant of leaf growth and so dry matter production in cassava. Ontogenic variation in NRA levels in cassava cultivars and hybrids might be further investigated as a possible selection tool.

Harper *et al* (1972) and Eck *et al* (1975) established differences in NRA in a range of soybean and sorghum hybrids respectively and Hageman *et al* (1967) demonstrated that NRA levels in maize could be manipulated by hybridization. Evidence was also here presented for possible genetic differences in NRA between the two cassava cultivars studied. Therefore if genetic differences between cassava cultivars could be firmly established, then optimum enzyme levels for highest leaf growth rates could be sought in an ideal cultivar.

Maximum NRA decreased with plant ontogeny in 1976 and 1977 crops with the

major reduction taking place between 36 and 63 days after planting. In experiment II, individual leaf NRA was found to reach maximum level prior to tuber initiation and then decline with increasing plant age. A reduction in canopy NRA occurred at the same time, also before obvious tuber development. MBS, which showed the highest NRA, also showed the smallest tuber dry weights in the measured period. These results were considered to indicate the close relationship between NRA and the changing pattern of assimilate distribution during plant ontogeny and tuber development in cassava.

Reductions in specific NRA with increasing plant age have also been reported in cereals and legumes (Wallace and Pate, 1967; Harper and Hageman, 1972). However, total plant canopy NRA increased in these crops to maximum levels which coincided with grain and podfill or the onset of the reproductive period (Harper and Hageman, 1972; Dalling *et al* 1975; Franco *et al,* 1977). Total NRA has been related to final grain and protein yields, although factors of nitrate availability and nitrogen translocation were also shown to be important (Jones and Sheard, 1977). Therefore it was felt that enzyme levels or activities fluctuated in response to the metabolic demands in these crops. A similar conclusion might be drawn from the results obtained with the cassava cultivars here studied.

From the results presented in this chapter, variation in NRA appeared to be in response to metabolic and physiological changes in the cassava leaf and plant. These were indicative of a switch from a predominantly nitrogen metabolism, favouring leaf and shoot growth to a carbohydrate metabolism favoring assimilate distribution to the root. This change took place at the time of tuber initiation and development and so might be in response to either increased demand for carbohydrate from the developing tuber sink or an alteration in the plant hormone balance preceding tuber initiation (Wilson *et al,* 1973). However, irrespective of how the switch occurred the decreased NRA should have released fixed carbon which might have otherwise been used for amino acid synthesis in the leaves for carbohydrate storage in the root tubers. This would be an advantage for high yield production in the cassava species (Wilson, 1975).

In summary, high nitrogen application stimulated shoot and leaf growth in the 2 cultivars. Variations in NRA during leaf ontogeny were described in the 2 cultivars, which were related to final leaf size. It was concluded that there was evidence for a significant reduction in NRA both within individual leaves and in the canopy, at the time of tuber initiation. This coincided with a reduction in the rate of shoot growth, which was felt to be indicative of a change in the pattern of assimilate production and translocation. There were also consistent and significant differences in NRA between the 2 cultivars.

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Table 1. Effect of N-supply on average rate of leaf production in two cassava cultivars over two experiments (1976, 1977).

TREATMENTS	Average rate of leaf production ($n.p.^{-1} . d^{-1}$.) (1)	
	1976	1977
Cultivars		
MBS	0.45	0.41
WS2	0.55	0.51
N. Supply		
N1	0.45	0.44
N2	0.52	0.48
Experiment Mean	0.47	0.46

(1) Average rates are calculated by regression analysis.

Table 2. Effect of N-supply and plant ontogeny on lamina area of mature leaves in two cassava cultivars

		Lamina area (cm^2)					
		Plant Ontogeny 36 DAP(1)			91 DAP		
		N1	N2	Mean	N1	N2	Mean
	MBS	173.9	194.0	184.0	91.2	142.4	116.8
1976	WS2	133.7	144.6	169.3	91.7	114.7	103.2
	Mean	153.8	169.3	161.6	91.5	128.6	110.0
	MBS	Plant Ontogeny 62 DAP			112 DAP		
	MBS	75.5	130.9	103.2 (a)	109.3	131.5	120.4 (a)
	WS2	48.5	39.4	44.5 (b)	86.8	98.8	92.3 (a)
	Mean	65.6 (a)	85.2 (a)	73.8	98.1 (a)	115.2 (a)	106.6
	S. E.		13.5			15.4	

(1) DAP: Days after planting to leaf appearance

Table 3. Effect of N-supply and plant ontogeny on lamina fresh weight of mature leaves in two cassava cultivars

	Lamina F. Wt. (g.) (27 days after leaf appearance)					
	Plant Ontogeny: 36 DAP(1)			91 DAP		
	N1	N2	Mean	N1	N2	Mean
MBS	2.5	2.9	2.7	1.1	1.7	1.4
1976 WS2	2.0	2.1	2.1	1.4	1.7	1.5
Mean	2.3	2.5	2.4	1.3	1.7	1.5
S.E. ±	0.3		0.2	0.3		0.2
	Plant Ontogeny: 62 DAP			112 DAP		
MBS	1.4	2.3	1.9(a)	1.4	2.0	1.7 (a)
1977 WS2	1.0	0.9	1.0(b)	1.4	1.4	1.4 (a)
Mean	1.2 (a)	1.6 (a)	1.5	1.4 (a)	1.7 (a)	1.6
S.E. ±	0.2			0.2		

(1) DAP: Days after planting to leaf appearance

Table 4. Reduction in maximum and average leaf nitrate reductase activities (NRA) during plant ontogeny in two experiments (1976, 1977)

Plant Ontogeny (DAP)	Nitrate reductase activity (NRA) ($nMNO-_2/g.\ f.\ wt.^{-1}/hr.^{-1}$	
	Maximum	Average
36 (1976)	5.71	4.48
63 (1977)	0.43	0.18
91 (1976)	0.28	0.23
112 (1977)	0.24	0.19

Table 5. Correlations between maximum leaf nitrate reductase activities (NRA), versus leaf size and leaf weight in cassava.

	Correlation Coefficient	Regression equation
NRA(x)VS – Leaf Weight (W)		
1976	0.94***	W = 0.375x +1.48
1977	0.38ns	–
NRA(x)VS – Leaf Area (A)		
1976	0.87***	A = 16.1x + 100.23
1977	0.28ns	–

ns: not significant
**: r = 0.01
***: r = 0.001

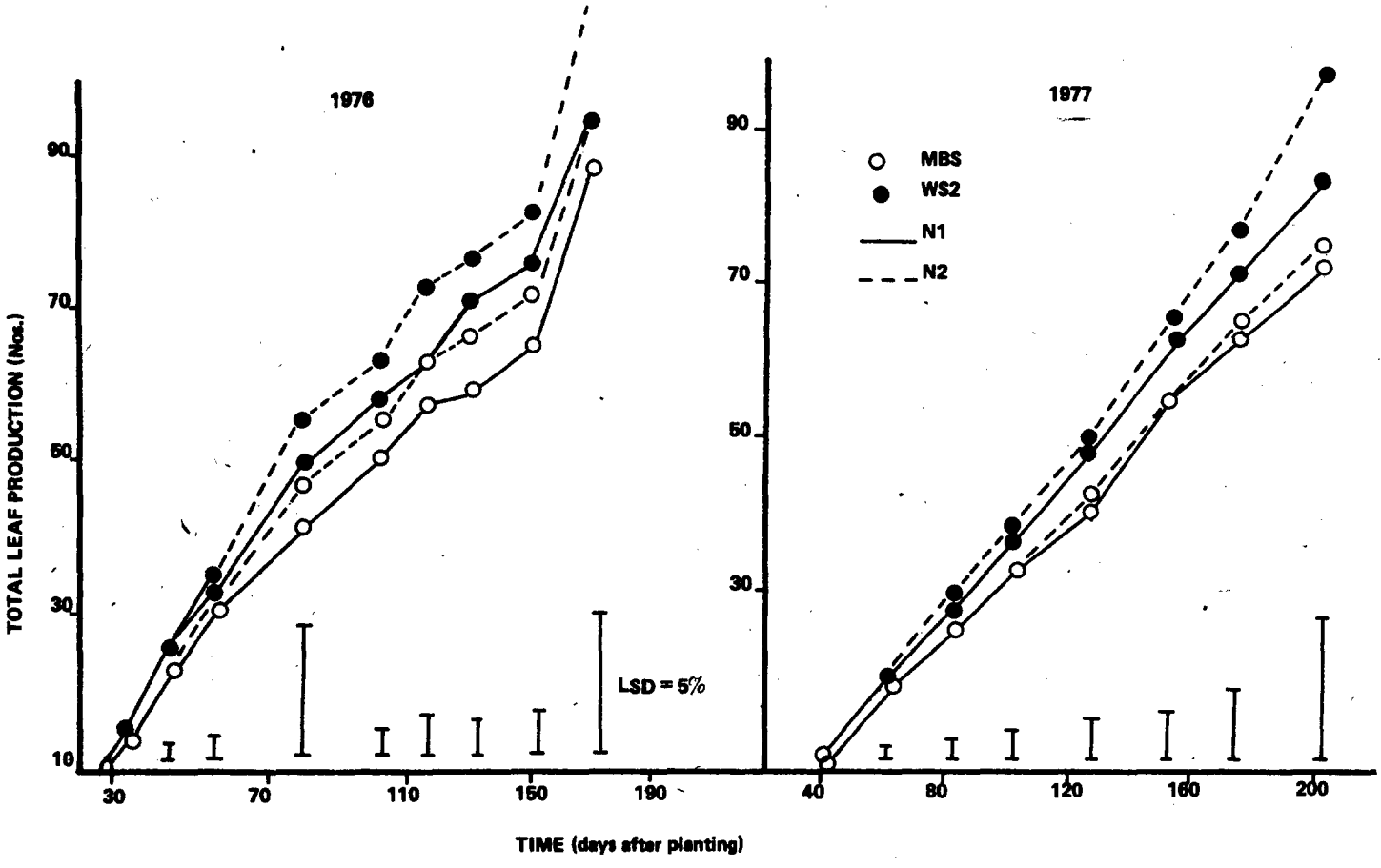

Figure 1. Effect of nitrogen on total leaf production during plant ontogeny in two cassava cultivars, grown in 1976 and 1977

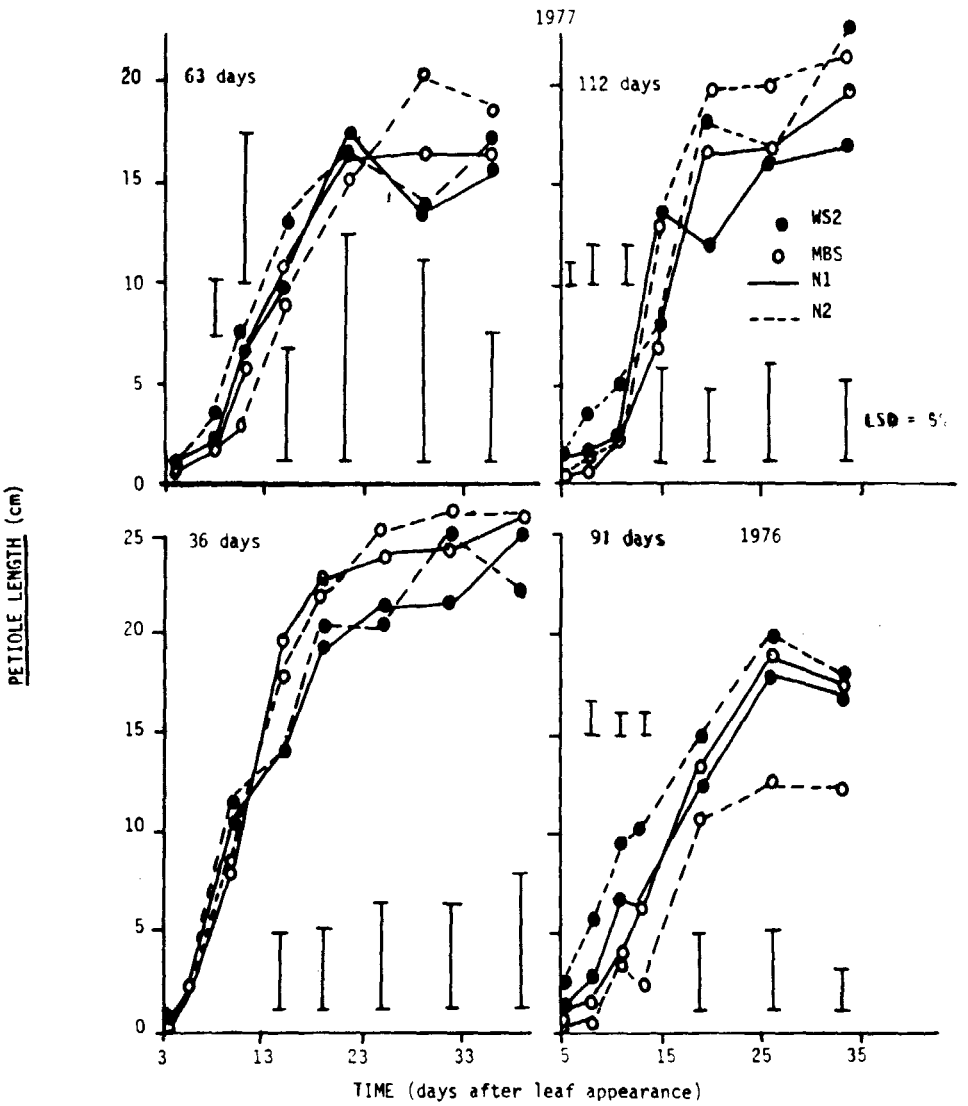

Figure 2. Effect of nitrogen on petiole length development in leaves appearing at two states of plant ontogeny of two cassava cultivars, grown in 1976 and 1977.

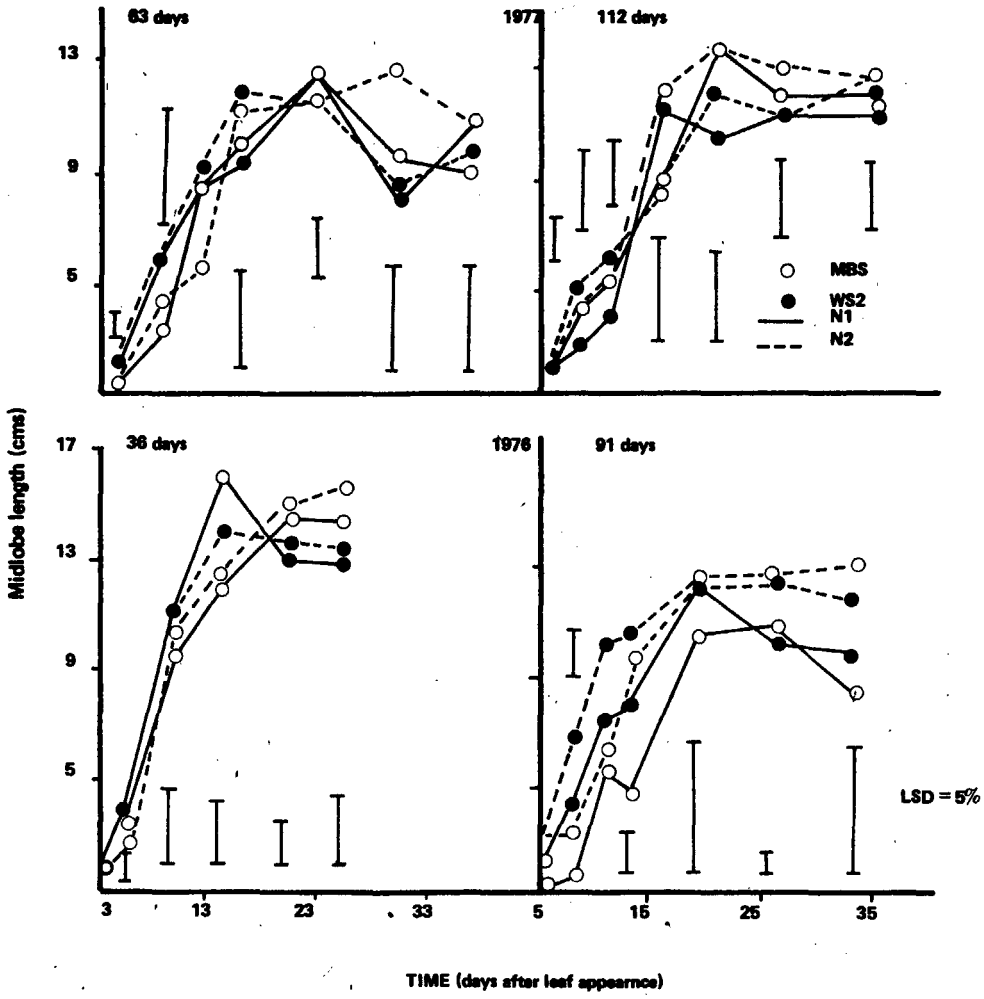

Figure 3. Effect of nitrogen on lamina midlobe length development in leaves appearing at two stages of plant ontogeny in two cassava cultivars, grown in 1976 and 1977

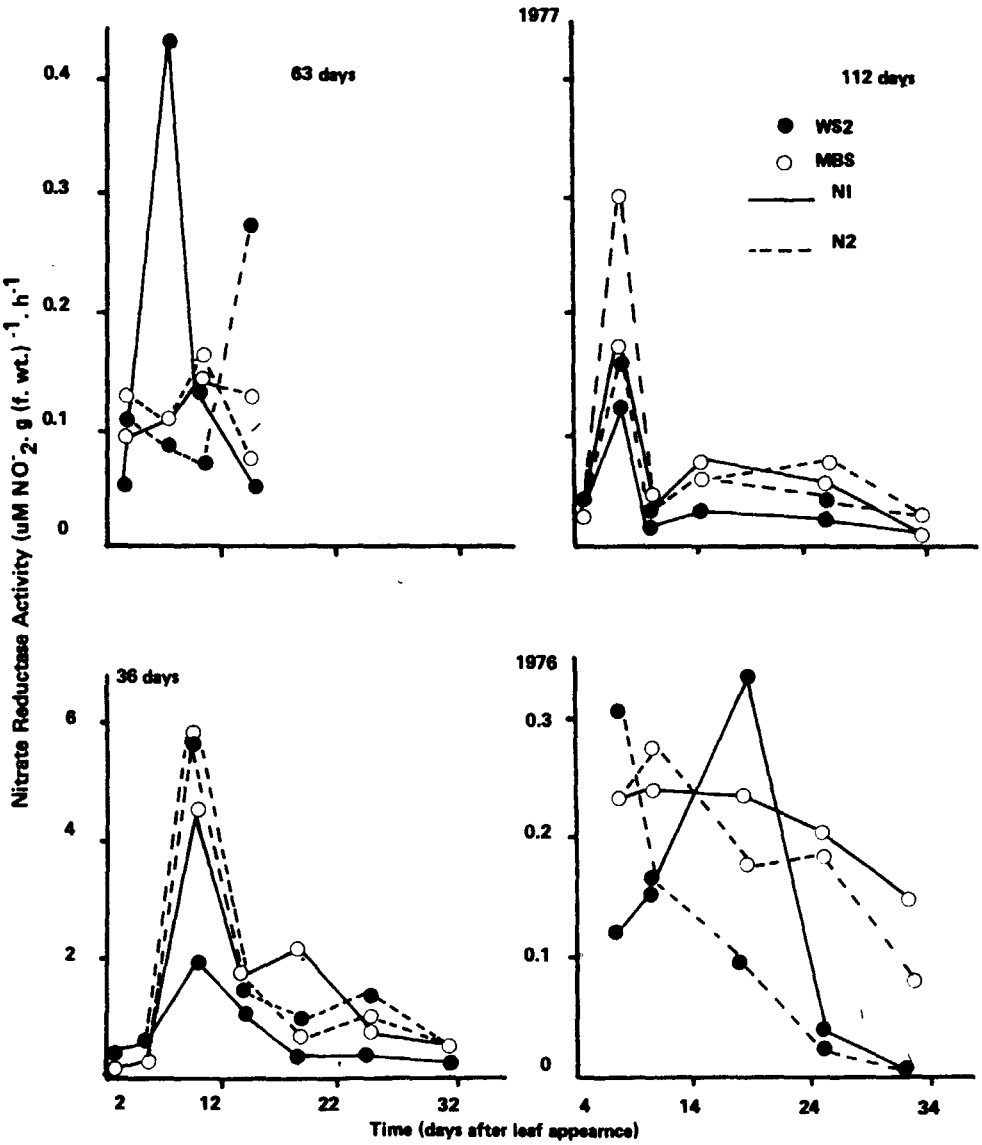

Figure 4. Effect of nitrogen on *in vivo* nitrate reductase activity in leaves appearing at two stages of plant ontogeny in two cassava cultivars, grown in 1976 and 1977

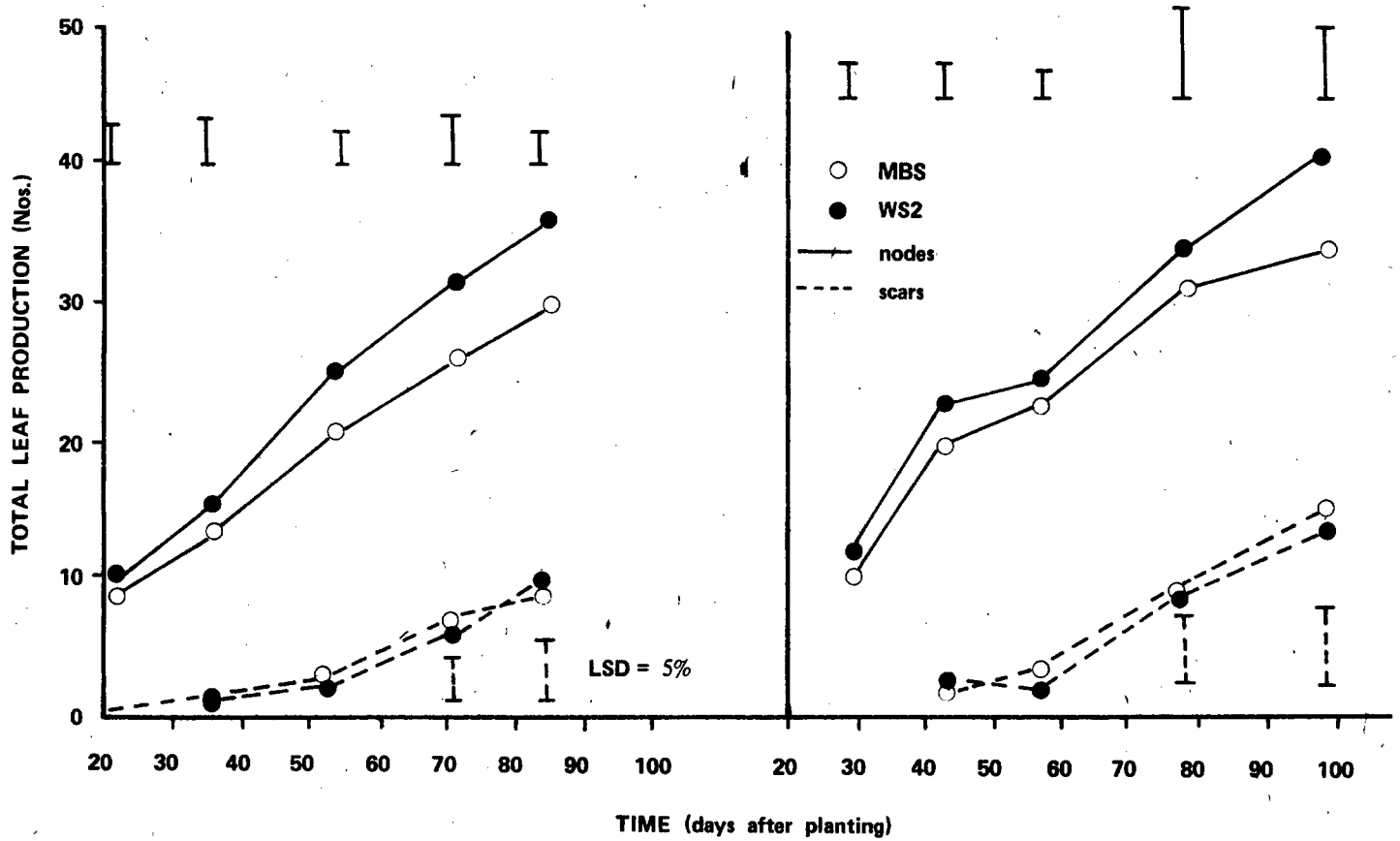

Fig. 5. Changes in total leaf production and leaf loss during plant ontogeny in two cassava cultivars.

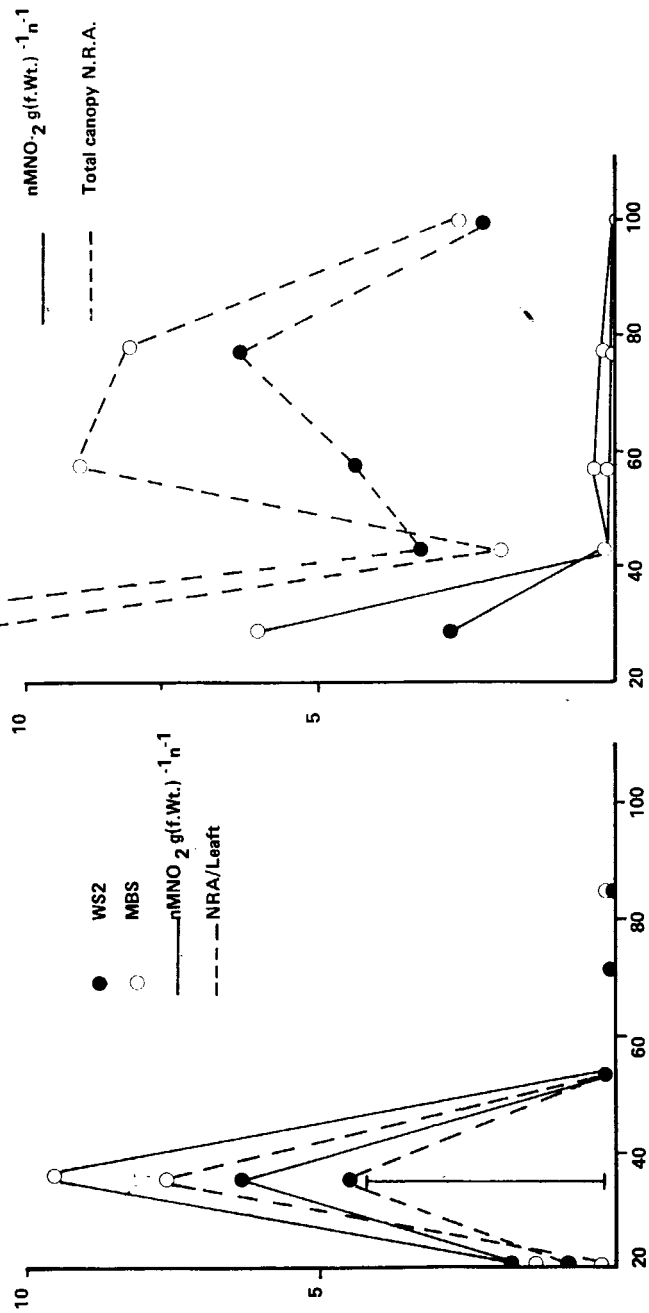

Figure 6. Changes in *in vivo* Nitrate reductase activity in the 7th leaf during plant ontogeny of two cassava cultivars

Figure 7. Changes in *in vivo* Nitrate reductase activity of the shoot canopy during plant ontogeny in two cassava cultivars