

EFFECTS OF NITROGEN AND CALCIUM ON THE LEVEL OF RESISTANCE OF CASSAVA TO THE MEALYBUG *P. MANIHOTI*

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Abstract—The effects of various levels of cow manure (equivalent to 0–50 kg nitrogen/ha.) or ground limestone (equivalent to 0–5 tons calcium/ha.) on the expression of resistance of cassava, *Manihot esculenta* Crantz to the cassava mealybug, *Phenacoccus manihoti* Mat. Ferr., were investigated under semi-controlled conditions in the laboratory. The study was made on two varieties of cassava, Moudouma and MM79, characterized by different levels of resistance in terms of antibiosis and tolerance. The antibiotic component was evaluated by determining the intrinsic capacity for increase r_c and tolerance by quantifying morphological and physiological modifications in the growth of plants induced by 2 months infestation by 100 mealybugs.

The antibiotic resistance of the two varieties was reduced by both fertilizers. However, the reduction was only significant at levels of 10 and 20 kg N/ha. and 1 and 1.5 t Ca/ha. for Moudouma and MM79 respectively.

In both varieties, tolerance was increased by the addition of nitrogen and not modified by the addition of lime.

The integration of these results into traditional cassava crops systems has also been discussed.

Key Words: *Phenacoccus manihoti*, cassava, insect-plant relationship, antibiosis, nitrogen, tolerance, lime, varietal resistance, Pseudococcidae

Résumé—L'influence de différents niveaux de fertilisation azotée (de 0 à 50 KgN/ha) ou calcique (de 0 à 5 tonnes/ha) sur l'expression de la résistance du manioc, *Manihot esculenta* Crantz, vis à vis de la cochenille farineuse du manioc, *Phenacoccus manihoti* Matt. Ferr., a été étudiée en conditions expérimentales de laboratoire. L'étude a porté sur deux variétés de manioc, Moudouma et MM79, caractérisées par des niveaux de résistance de type antibiose et tolérance différents. La composante antibiotique a été appréciée au travers de la détermination de la capacité intrisèque d'accroissement r_c et, la tolérance en quantifiant les modifications morphologiques et physiologiques de la croissance des plantes provoquées par une infestation de 2 mois par cent cochenilles.

La résistance antibiotique des deux variétés de manioc est réduite par les deux fertilisations minérales. La réduction n'est cependant significative qu'aux taux 10 et 20 kgN/ha. respectivement pour Moudouma et MM79 et, aux taux 1 et 1,5 T/ha. de calcaire pour les deux variétés.

Chez les deux variétés, la résistance de type tolérance est augmentée par l'apport d'azote et non modifiée par l'apport de calcaire.

L'intégration de ces résultats, dans les systèmes culturaux traditionnels à base de manioc est par ailleurs évoquée.

INTRODUCTION

Different levels of resistance of cassava, *Manihot esculenta* Crantz. Euphorbiaceae to the mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hom. Pseudococcidae), namely antibiosis and tolerance, have been identified and quantified during varietal selection experiments performed in the field and in the laboratory (Le Rü et al., 1991).

A knowledge of the influence of environmental factors (temperature, relative humidity, soil fertility) on the induced resistance (temporary) of plants may be valuable as a complement to the genetic resistance in the control of insect pests (Tingey and Singh, 1980). Among the many environmental factors that man can modify fairly easily are the edaphic characteristics.

Field trials were performed between 1986 and 1990 in different localities in South Congo, particularly in the region of Bouenza, in order to determine the optimal growth conditions for cassava (Arrivets, 1989). They showed that the addition, of ground limestone at the rate of 500 kg/ha. in association with either NPK fertilizer (urea: 150 kg/ha. or 45 kg P_2O_5/ha.; potash: 50 kg K_2O/ha.) or with 30 tons manure/ha. (45 kg N/ha.) could increase the yield of cassava tubers significantly. These results confirm the positive effect of the nitrogen fertilizer on the yields of cassava tubers reported several times previously (Cock and Reyes, 1985; Howeler, 1985; Njoku and Odurukwe, 1987). Tolerance can be defined as the capacity of some plants to grow despite signs of damage by high levels of insect pests, which would normally cause severe damage in other non-tolerant plants (Painter, 1951). Current crop practices, whilst aiming to increase yields, also tend to increase plant tolerance.

However, the addition of fertilizers can greatly modify the nutritional value of the plant and its contents of allelochemicals and also influence its antibiotic resistance to attacks by phytophagous insects (Rodriguez, 1960; Harrewijn, 1977; Harrewijn and Dieleman, 1984). In general, nitrogen appears to favour the multiplication of the Coccoidea (Thompson, 1941; Fennah, 1959; Salama et al., 1972; Smirnoff and Valero, 1975; McClure, 1980; Campbell, 1984; Washburn et al., 1987).

The effect of lime has not been studied by many workers. Steyn (1951) has showed that lack of calcium increased the sensitivity of *Citrus* towards *Aonidiella aurantii* Mask (Hom. Diaspididae) and Nadir (1965) reported that an excess of calcium increased their multiplication. The only work relating to the fecundity of *P. manihoti* is that of Lema and Mahungu (1983) who reported that fecundity was increased by an addition of 60 kg N/ha.; these authors nevertheless remarked that the method they employed was not conclusive.

Previous data, however suggest that the use of fertilizers such as manure and lime could decrease antibiosis and increase tolerance of cassava to *P. manihoti*. The aim of our work was to define the influence of different levels of N or Ca on the expression of resistance (antibiosis and tolerance) in two varieties of cassava to *P. manihoti* under laboratory conditions.

The antibiotic component was assessed by determining the intrinsic capacity for increase r_c (Laughlin, 1965) which is a good estimator of this category of resistance (Sumner et al., 1986).

Tolerance was evaluated by quantifying changes in parameters (leaf area, dry weight, leaf area ratio $\bar{F}$, relative growth rate $\bar{R}$ and net assimilation rate $\bar{E}$), induced by pest infestation and affecting the yield; the latter is strongly influenced by leaf area and growth rate (Schulthess et al., 1991). Their quantification provides a useful tool to assess the degree of plant tolerance (Scott et al., 1991: Webster et al., 1991).

MATERIALS AND METHODS

The study was performed on two varieties of cassava giving high yields in Congo (up to 25 t/ha.), Moudouma and MM79. Moudouma was chosen because it is the most commonly grown variety in the Bouenza region and also because it has been used as the reference variety in all field trials done between 1986 and 1990 in South Congo (Arrivets, 1989). The variety MM79 is a selected variety judged by the selection committee of the Cassava National Programme of Congo to be one of the most promising varieties (Mabanza, Pers. commun.). In addition, these two varieties have significantly different resistances in terms of antibiosis (r_c = 0.143 and 0.155 for Moudouma and MM79, respectively) (Le Rü et al., 1991) and tolerance. MM79 being more tolerant than Moudouma (Tertuliano, Pers. commun.).

Plants were obtained from 30 cm cuttings planted vertically with two-thirds of their length in soil held in plastic pots (40 x 28 cm). Pots were left in the shade for 2 weeks to stimulate the rooting process. Thereafter, they were placed in full light until the 9–10-leaf-stage (about 70 cm in height), which took 9–10 weeks. Pots were then left for a further 2 months in a growth chamber under semi-controlled conditions with an average temperature of 25°C (minima-maxima 21–32°C) and an average 70% r.h. (ranging between 60 and 80%); photoperiod was 12 h L:D. Under these experimental conditions, we obtained, after 4 months period, plants with approximately 20–25 leaves and about 100–110 cm in height. Under natural conditions in Congo, attacks by mealybugs occur at the beginning of the dry season on plants 3–8 months old, derived from the propagation of cassava cuttings, mostly performed between November and April. Pots were watered twice per week during the experiment. For each variety, we studied the effect of nitrogen (cow manure) as a fertilizer and calcium (ground limestone from Madingou), using a control (C) and 5 levels D_1 to D_5 (10 to 50 t / ha. of cow manure equivalent to 15 to 75 kg N/ha.) and, 0.5 to 2.5. t lime/ha. The levels of fertilizers used fell in the range of levels used by Arrivets (1989) during field trials, i.e. 0.5–5 tons ground limestone/ha. and 40–80 kg N/ha. The addition of lime and manure was carried out before cuttings were made, as it was realized during field trials by Arrivetz (1989).

The strain of *P. manihoti* (a species reproducing by thelytocous parthenogenesis) used for these experiments was reared on the M'Pembe variety.

The antibiotic component of resistance was determined by measuring the daily intrinsic capacity for increase r_c (r_c = Log T_c /R_o with T_c = mean generation time in days and R_o = net reproduction rate) of Laughlin (1965) on five cohorts of 100 mealybugs for each treatment. This parameter which is a good estimator of antibiotic resistance of plants (Sumner et al., 1986), integrates the different development components of the insect (duration of development and larval mortality, survival rate and daily fecundity).

Tolerance was determined by quantifying changes in leaf area, dry weight, leaf area ratio ($\bar{F}$), relative growth rate ($\bar{R}$) and net assimilation rate ($\bar{E}$) of the plant induced by pest infestation. At time 0 (T_1), 15 plants per levels of fertilizer, of uniform appearance, without symptoms of African Cassava Mosaic Virus, were chosen among the cuttings prepared initially. These were divided randomly into two groups: one group of five plants, which was harvested immediately (T_1) and one group of five pairs of plants which was harvested 2 months later (T_2). Each pair consisted of an uninfested plant (without mealybug) and an infested plant (infested with 100 neonate mealybugs, L_1 larvae less than a day old). These conditions of infestation (100 mealybugs per plant for 2 months) were representative of the maximum number usually observed 2 months after the beginning of gradation in Congo (Le Rü et al., 1991b). The artificial infestation is done on the second and third leaves from the apex with young neonate larvae. They attach themselves to the plant within the first 48 hr, resulting in an homogeneous infestation of plants by *P. manihoti*. We then count and readjust their number to 100 (by removing or adding mealybugs of the same stage) during all the experiments, in order to have an identical number on all infested plants. Finally, when mealybugs reach the L_4 stage with ovisacs, we remove the ovisacs each week to prevent new larvae from hatching and to keep the total number constant. The following parameters were determined at times T_1 and T_2 on control and experimental plants for each host-plant: the mean leaf area and the total plant dry weights. Leaf areas were determined using the equation: Log (LA) = -7.47 + 2.460 $\log_e$(MLL) (LA = leaf area in cm²; MLL = median lobe length in cm) established for different aged leaves and different cultural conditions (Hammer, 1980). Dry weights were measured after drying the plant material in the oven at < 50°C for 4 days.

From these parameters, we determined three indices $\bar{R}$, $\bar{E}$ and $\bar{F}$ which were used to characterize the physiological state of a plant. The relative growth rate $\bar{R}$ for a period of time can be expressed as:

$$\bar{R} = (L_n\, W_2 - L_n\, W_1/(t_2 - t_1)$$

where W_1 and W_2 are the total dry weights where beginning (T_1) and end (T_2) of the time period; R indicates the variation in plant weight per unit of time.

The rate of net assimilation $\bar{E}$ is:

$$\bar{E} = (W_2 - W_1/(t_2 - t_1)\, Ln\, A_2 - Ln\, A_1)/(A_2 - A_1)$$

where A_1 and A_2 are the leaf areas at times T_1 and T_2 respectively; $\bar{E}$ is an approximate measure of the net photosynthetic rate (Causton and Venus, 1981). In a constant environment, $\bar{E}$ can be considered as an index of the plants productive efficiency (Williams, 1946).

The leaf area ratio $\bar{F}$ is:

$$\bar{F} = (A_1/W_1) + (A_2/W_2/2)$$

It is a morphological index of the plant and it assumes that leaves are the sole assimilatory organs (Causton and Venus, 1981).

For analysis of variance, factors "variety" (Moudouma and MM79), "treatment" (Manure and lime), "infestation" (uninfested and infested) and "levels" (Control, D_1, D_2, D_3, D_4, D_5) were considered as a fixed model. Leaf areas, dry weights, relative growth rates, rates of net assimilation and leaf area ratios were found to be homogeneously distributed between factors, as tested with the Kolmogorov Smirnov test (homoscedasticity hypothesis for ANOVA). Within each significant infestation factor (P < 0.05) means were compared using Fisher's PLSD multiple range test; mean values ± SE bearing identical letters are not significantly different at the 5 % level. Mean values of R_o and r_c were analysed using a t test at the 5% level. These statistics were completed using the Statview software (Abacus Concept, USA).

RESULTS AND DISCUSSION

The antibiotic resistance of the two cassava varieties to *P. manihoti* was reduced by both mineral fertilizers. However, the reduction was only significant at levels D_1 and D_2 of manure for Moudouma and MM79 respectively, and at levels D_2 and D_3 of lime for both varieties (Table 1). Net reproduction rate (R_o) increased with addition of both fertilizer, especially at D_2 level, although this was never significant. Throughout the range of fertilizer treatment, we observed that Moudouma was always more resistant than MM79 (r_c Moudouma < r_c MM79), even though the difference was no longer

Table 1. Means of net reproduction rate (R_o) and the intrinsic capacity for growth (r_c) of the cassava mealybug, *P. manihoti*, reared on cassava varieties Moudouma and MM79, for different levels of cow manure or ground limestone (Control D_1, D_2, D_3, D_4 and D_5).

Levels	Manure				Lime			
	Moudouma		MM79		Moudouma		Zanaga	
	R_o	r_c	R_o	r_c	R_o	r_c	R_o	r_c
Control	322.3 ± 35.4 a	0.141 ± 0.003 a	352.8 ± 43.2 a	0.154 ± 0.005 a	322.3 ± 35.4 a	0.141 ± 0.003 a	352.8 ± 43.2 a	0.154 ± 0.005 a
D_1	375.5 ± 42.8 a	0.148 ± 0.002 b	372.1 ± 36.8 a	0.159 ± 0.004 ab	359.6 ± 36.8 a	0.148 ± 0.004 ab	392.3 ± 35.2 a	0.162 ± 0.006 ab
D_2	385.2 ± 38.7 a	0.147 ± 0.004 ab	432.8 ± 42.1 a	0.165 ± 0.004 b	410.2 ± 41.5 a	0.154 ± 0.004 b	446.0 ± 38.6 a	0.179 ± 0.006 b
D_3	361.4 ± 41.9 a	0.145 ± 0.004 ab	374.7 ± 51.0 a	0.160 ± 0.003 ab	397.4 ± 42.3 a	0.152 ± 0.006 b	410.8 ± 43.7 a	0.172 ± 0.004 b
D_4	355.2 ± 36.2 a	0.142 ± 0.003 ab	342.8 ± 42.8 a	0.153 ± 0.004 ab	361.7 ± 38.7 a	0.149 ± 0.005 ab	379.2 ± 32.0 a	0.161 ± 0.006 ab
D_5	336.4 ± 36.7 a	0.143 ± 0.003 ab	326.9 ± 40.3 a	0.150 ± 0.005 ab	354.9 ± 35.0 a	0.148 ± 0.004 ab	371.7 ± 41.2 a	0.162 ± 0.005 ab

significant at the level D_5 of manure. The variation in resistance was more marked in the lime treatment and was greater in MM79. MM79 r_c varied with 7% (manure) and 16% (lime) and r_c Moudouma of 5% (manure) and 9 % (lime).

The present work shows that the level of antibiotic resistance is reduced by the addition of fertilizers such as nitrogen and calcium. The change in the plant's response with the level is slightly different, depending on the fertilizer used and the variety. Our results agree with those of Lema and Mahungu (1983) who reported a higher fecundity in *P. manihoti* when nitrogen was added to the soil. Results also confirm the various works done on mealybugs which have reported a decrease in resistance with the addition of nitrogen (Smirnof and Valero, 1975; McClure, 1980; Campbell, 1984; Washburn et al., 1987; Beattie et al., 1990) or calcium (Nadir, 1965; Chaboussou, 1974). In general, it is recognized that calcium fertilization favours the assimilation of nitrogen by plants (Soltner, 1985) and most authors mentioned above agree that the addition of nitrogen and calcium results in an increase in the nitrogenous compounds of the plants, which enhances insect feeding (McNeil and Southwood, 1978). *P. manihoti* is a phloem-feeding insect (Calatayud, 1993) and thus we can speculate that a change in its biotic potential has its origins in a modification of the sap composition of cassava. By analogy with plant aphid models, by far the best studied (Auclair, 1989), this modification is thought to take place via three different mechanisms or a combination of these (Harrewijn and Dieleman, 1984), modification in

(i) the quantity of sap available to the insect.

(ii) the quality and quantity of nutrients (essentially amino acids and carbohydrates).

(iii) allelochemicals present in the sap (deterrent and toxic, in particular phenols, the only secondary substances in cassava, whose presence in phloem sap has been demonstrated (Calatayud, 1993).

It must be emphasized that our results disagree with those obtained in Nigeria and Benin (Neuenschwander et al., 1990) and in Ivory Coast and Ghana (Neuenschwander et al., 1989). The conclusions were that the largest numbers of mealybugs were recorded on poor soils (in terms of organic matter), particularly on sandy soils in forest zones. Such a comparison is difficult to make since our study conditions were very different from field conditions (plants in pots, varieties, soil) and differences observed may be due to some other factor than nitrogen uptake by the plant.

The level of tolerance is increased by nitrogen, but not modified by calcium. The leaf area and total dry weight of the two cassava varieties increased

Table 2. Means ($n = 5$) of leaf area of two cassava varieties Moudouma and MM79, uninfested (ui) or infested (i), for different levels of cow manure or ground limestone (Control D_1, D_2, D_3, D_4 and D_5), and results of 4-way ANOVA (variety, treatment, infestation and level)

		Manure		Lime	
Levels	Infestation	Moudouma	MM79	Moudouma	MM79
Control	ui	97.45 ± 12.36 a	83.50 ± 12.60 a	97.45 ± 12.36 a	83.50 ± 12.60 a
	i	67.28 ± 14.57 b	64.70 ± 6.50 b	67.28 ± 14.57 b	64.70 ± 6.50 b
D_1	ui	123.0 ± 16.70 a	88.60 ± 13.60 a	106.32 ± 15.63 a	97.58 ± 14.21 a
	i	83.50 ± 11.36 b	68.90 ± 9.56 b	75.29 ± 14.91 b	69.54 ± 10.24 b
D_2	ui	136.20 ± 22.9 a	18.0 ± 12.60 a	102.32 ± 12.25 a	103.82 ± 13.51 a
	i	89.40 ± 10.40 b	58.30 ± 10.97 b	71.41 ± 13.58 b	75.36 ± 13.70 b
D_3	ui	139.20 ± 20.70 a	108.40 ± 15.10 a	103.47 ± 13.35 a	107.23 ± 17.50 a
	i	92.30 ± 12.71 b	79.0 ± 11.43 b	75.23 ± 13.63 b	77.53 ± 12.80 b
D_4	ui	140.10 ± 21.30 a	131.10 ± 14.70 a	105.56 ± 12.84 a	100.34 ± 11.85 a
	i	96.40 ± 10.23 b	96.0 ± 13.90 b	72.45 ± 14.38 b	71.61 ± 13.79 b
D_5	ui	138.90 ± 17.24 a	148.20 ± 18.50 a	89.72 ± 13.26 a	111.29 ± 15.60 a
	i	100.10 ± 9.41 b	104.60 ± 14.70 b	58.43 ± 14.27 b	76.45 ± 15.64 b

Factors of ANOVA P (Analysis of variance)

Variety (A)	0.0065	Level (D)	0.0098
Treatment (B)	0.0171	AD	0.02341
AB	0.0365	BD	0.03512
Infestation (C)	0.0109	ABD	0.0569
AC	0.0096	CD	0.0535
BC	0.0848	ACD	0.4628
ABC	0.0513	BCD	0.8315
		ABCD	0.7581

Table 3. Means ($n = 5$) of total dry weights of two cassava varieties Moudouma and MM79, uninfested (ui) or infested (i), for different levels of cow manure or ground limestone (Control D_1, D_2, D_3, D_4 and D_5) and results of 4-way ANOVA (variety, treatment, infestation and level)

		Manure		Lime	
Levels	Infestation	Moudouma	MM79	Moudouma	MM79
Control	ui	6.25 ± 0.96	6.22 ± 1.25	6.25 ± 0.96	6.22 ± 1.25
	i	5.34 ± 0.53	5.68 ± 0.85	5.34 ± 0.53	5.68 ± 0.85
D_1	ui	7.52 ± 0.87	6.06 ± 1.24	7.13 ± 0.68	7.12 ± 0.84
	i	6.37 ± 0.93	5.02 ± 0.75	6.71 ± 0.48	5.62 ± 0.71
D_2	ui	7.21 ± 1.34	6.70 ± 1.38	6.93 ± 0.63	7.52 ± 0.92
	i	6.06 ± 0.45	5.86 ± 0.83	6.22 ± 0.57	6.34 ± 0.83
D_3	ui	7.77 ± 0.98	7.90 ± 1.56	7.25 ± 0.74	7.43 ± 0.76
	i	6.69 ± 0.51	6.34 ± 0.79	6.54 ± 0.43	6.41 ± 0.70
D_4	ui	9.30 ± 1.11	9.40 ± 1.55	7.41 ± 0.40	6.92 ± 0.86
	i	7.80 ± 0.82	8.25 ± 1.14	6.78 ± 0.81	5.50 ± 0.85
D_5	ui	9.53 ± 0.95	9.34 ± 1.37	7.01 ± 0.84	7.40 ± 0.96
	i	8.44 ± 1.24	8.56 ± 1.43	6.03 ± 0.36	5.84 ± 0.78

Factors of ANOVA P (Analysis of variance)

Variety (A)	0.9593	Level (D)	0.0001
Treatment (B)	0.0001	AD	0.3953
AB	0.116	BD	0.001
Infestation (C)	0.2588	ABD	0.3825
AC	0.8146	CD	0.3402
BC	0.831	ACD	0.951
ABC	0.2844	BCD	0.5424
		ABCD	0.6272

Table 4. Means (n = 5) of unit leaf area ($\bar{E}$) of two cassava varieties Moudouma and MM79, uninfested (ui) or infested (i), for different levels of cow manure or ground limestone (Control D_1, D_2, D_3, D_4 and D_5) and results of 4-way ANOVA (variety, treatment, infestation and level)

		Manure		Lime	
Levels	Infestation	Moudouma	MM79	Moudouma	MM79
Control	ui	0.272 ± 0.027	0.265 ± 0.018	0.272 ± 0.027	0.265 ± 0.018
	i	0.241 ± 0.023	0.261 ± 0.016	0.241 ± 0.023	0.261 ± 0.016
D_1	ui	0.323 ± 0.025	0.258 ± 0.026	0.276 ± 0.019	0.286 ± 0.019
	i	0.298 ± 0.021	0.242 ± 0.015	0.242 ± 0.024	0.264 ± 0.022
D_2	ui	0.317 ± 0.027	0.262 ± 0.017	0.295 ± 0.032	0.289 ± 0.016
	i	0.284 ± 0.020	0.249 ± 0.025	0.246 ± 0.024	0.266 ± 0.021
D_3	ui	0.311 ± 0.040	0.287 ± 0.021	0.291 ± 0.025	0.285 ± 0.018
	i	0.276 ± 0.028	0.263 ± 0.016	0.258 ± 0.037	0.253 ± 0.020
D_4	ui	0.357 ± 0.034	0.328 ± 0.017	0.302 ± 0.032	0.278 ± 0.015
	i	0.304 ± 0.024	0.303 ± 0.022	0.256 ± 0.017	0.247 ± 0.022
D_5	ui	0.342 ± 0.039	0.336 ± 0.024	0.283 ± 0.025	0.283 ± 0.024
	i	0.296 ± 0.037	0.327 ± 0.019	0.255 ± 0.032	0.260 ± 0.015

Factors of ANOVA P (Analysis of variance)

Variety (A)	0.0159	Level (D)	0.013
Treatment (B)	0.0135	AD	0.6945
AB	0.3133	BD	0.0261
Infestation (C)	0.0737	ABD	0.5169
AC	0.9902	CD	0.9235
BC	0.3173	ACD	0.9947
ABC	0.5014	BCD	0.9868
		ABCD	0.9887

Table 5. Means (n = 5) of leaf area ratio ($\bar{F}$) of two cassava varieties Moudouma and MM79, uninfested (ui) or infested (i), for different levels of cow manure or ground limestone (Control D_1, D_2, D_3, D_4 and D_5) and results of 4-way ANOVA (variety, treatment, infestation and level)

		Manure		Lime	
Levels	Infestation	Moudouma	MM79	Moudouma	MM79
Control	ui	0.161 ± 0.14 a	0.163 ± 0.012 a	0.161 ± 0.014 a	0.163 ± 0.012 a
	i	0.137 ± 0.009 b	0.139 ± 0.009 b	0.137 ± 0.009 b	0.139 ± 0.009 b
D_1	ui	0.152 ± 0.008 a	0.159 ± 0.013 a	0.164 ± 0.013 a	0.163 ± 0.014 a
	i	0.128 ± 0.016 b	0.132 ± 0.010 b	0.132 ± 0.011 b	0.150 ± 0.011 a
D_2	ui	0.165 ± 0.008 a	0.150 ± 0.016 a	0.160 ± 0.013 a	0.165 ± 0.017 a
	i	0.141 ± 0.014 b	0.142 ± 0.008 a	0.143 ± 0.14 a	0.149 ± 0.012 a
D_3	ui	0.157 ± 0.009 a	0.147 ± 0.013 a	0.151 ± 0.13 a	0.166 ± 0.013 a
	i	0.139 ± 0.016 a	0.124 ± 0.009 b	0.140 ± 0.015 a	0.148 ± 0.012 a
D_4	ui	0.163 ± 0.012 a	0.165 ± 0.015 a	0.157 ± 0.020 a	0.161 ± 0.009 a
	i	0.141 ± 0.009 b	0.138 ± 0.011 b	0.148 ± 0.019 a	0.147 ± 0.010 a
D_5	ui	0.161 ± 0.009 a	0.160 ± 0.010 a	0.152 ± 0.017 a	0.164 ± 0.013 a
	i	0.139 ± 0.011 b	0.141 ± 0.009 b	0.147 ± 0.16 a	0.157 ± 0.015 a

Factors of ANOVA P (Analysis of variance)

Variety (A)	0.01838	Level (D)	0.0392
Treatment (B)	0.02341	AD	0.4607
AB	0.0015	BD	0.1382
Infestation (C)	0.0186	ABD	0.0748
AC	0.5573	CD	0.0607
BC	0.0178	ACD	0.5015
ABC	0.9796	BCD	0.3549
		ABCD	0.4539

Table 6. Means ($n = 5$) of net relative growth rate ($\bar{R}$) of two cassava varieties Moudouma and MM79, uninfested (ui) or infested (i), for different levels of cow manure or ground limestone (Control D_1, D_2, D_3, D_4 and D_5) and results of 4-way ANOVA (variety, treatment, infestation and level)

		Manure		Lime	
Levels	Infestation	Moudouma	MM79	Moudouma	MM79
Control	ui	0.025 ± 0.003	0.024 ± 0.005	0.025 ± 0.003	0.024 ± 0.005
	i	0.023 ± 0.003	0.023 ± 0.004	0.023 ± 0.003	0.023 ± 0.004
D_1	ui	0.030 ± 0.004	0.023 ± 0.003	0.027 ± 0.004	0.025 ± 0.003
	i	0.026 ± 0.004	0.018 ± 0.005	0.023 ± 0.003	0.019 ± 0.004
D_2	ui	0.033 ± 0.003	0.023 ± 0.004	0.027 ± 0.003	0.027 ± 0.004
	i	0.027 ± 0.004	0.018 ± 0.004	0.022 ± 0.003	0.022 ± 0.004
D_3	ui	0.029 ± 0.004	0.030 ± 0.005	0.028 ± 0.004	0.026 ± 0.003
	i	0.024 ± 0.003	0.026 ± 0.003	0.023 ± 0.003	0.018 ± 0.003
D_4	ui	0.033 ± 0.004	0.035 ± 0.006	0.029 ± 0.004	0.026 ± 0.005
	i	0.029 ± 0.003	0.030 ± 0.005	0.022 ± 0.004	0.019 ± 0.003
D_5	ui	0.035 ± 0.005	0.033 ± 0.007	0.027 ± 0.003	0.026 ± 0.004
	i	0.030 ± 0.004	0.031 ± 0.005	0.020 ± 0.004	0.021 ± 0.003

Factors of ANOVA P (Analysis of variance)

Variety (A)	0.0794	Level (D)	0.0229
Treatment (B)	0.0098	AD	0.3861
AB	0.2562	BD	0.1253
Infestation (C)	0.1064	ABD	0.4311
AC	0.8978	CD	0.9999
BC	0.8689	ACD	0.9997
ABC	0.8689	BCD	0.9995
		ABCD	0.9997

significantly when manure was added (Tables 2 and 3). Both varieties responded differently. In Moudouma, the response was observed at the lowest level (D_1) and did not vary significantly with increasing levels. In MM79, a response was obtained at level D_3 only and increased until level D_5. In both varieties and whatever the level, the infestation by *P. manihoti* caused a significant reduction in leaf area ranging between 22 and 34%. A reduction in total dry weight of plants was also observed as a result of infestation, although this was not significant. Values of the relative growth rate, $\bar{R}$ and net assimilation rate $\bar{E}$ increased (from the lowest D_1 for Moudouma and from level D_3 onwards for MM79) whereas values of the leaf area ratio $\bar{F}$ remained unchanged with the addition of manure (Tables 4–6). We observed a reduction in the values of parameters $\bar{R}$, $\bar{E}$ and $\bar{F}$ of infested plants at all levels of manure in both varieties, although this was only significant for $\bar{F}$. In both varieties, none of the morphological and physiological parameters studied were modified by lime addition (Tables 4–6). By contrast, we observed a reduction (not significant) in these parameters measured in infested plants.

This work shows the beneficial effect of the nitrogen fertilizer on the tolerance of cassava to the mealybug, as seen by the increase in leaf area, total dry weight, $\bar{R}$ and $\bar{E}$ of plants in the presence of nitrogen despite infestation. The values of these parameters on infested plants were equal or greater than those of control non infested plants, when the level reached 10 and 30 kg N/ha. for Moudouma and MM79, respectively. A similar result was reported by Goergen and Neuenschwander (1992) on the cassava variety TMS 91934 grown with low and high levels of nitrogen, after infestation by *P. manihoti*. These results confirm observations made on aphids, i.e. an increase in plant tolerance is seen when nitrogen is added to the soil (Janson and Smilowitz, 1986; Ankersmit, 1989; Riedell, 1990). Our experiment has not shown that calcium had an effect on cassava tolerance nor any effect on growth (none of the parameters studied here were modified significantly in non-infested plants). The beneficial effect of calcium on the growth of plants, reported by Arrivets (1989) in field trials, may be due to its association with added nitrogen, which stimulates its assimilation by the plant ; it remains to be verified if a low level of calcium (< 10 t/ha. combined with a low level of nitrogen (< 15 kg/ha.), gives an improvement as important as nitrogen alone (> 45 kg N/ha.) on the expression of tolerance in cassava to the mealybug.

This study also suggests that the addition of manure at a rate of 60–75 kg N/ha. could be used advantageously to increase the resistance of cassava to *P. manihoti* at this level, antibiosis is not modified and tolerance is increased. Preliminary field trials should be carried out to verify these results; the expression of induced resistance might be different depending on environmental conditions (Tingey and Singh, 1980). Results also indicate that adding manure has an antagonistic effect on the antibiotic and tolerance components of cassava to *P. manihoti*. Ankersmith (1989) has pointed out that concerning the use of nitrogen within the framework of integrated pest control against cereal aphids, it seems difficult to find a good compromise between high yields (increase of tolerance) and high pest populations (decrease of antibiosis). The integration of these data in a simulation model such as the one developed by Gutierrez et al. (1988) could bring some answers. However beforehand, it will be necessary to study the effect of nitrogen on the third component of resistance in cassava, antixenosis. Harrewijn and Piron (1988) have shown on the model lettuce-aphid (*Lactuca sativa* L. Compositae/*Nasonovia ribisnigi* Mosley, Aphididae) that adding manure increased antixenosis and decreased antibiosis in the plant. The high level of antixenosis expressed by some varieties of cassava (Tertuliano, Pers. commun.) tends to suggest that when the mealybug attaches itself to the plant, and in particular during the penetration of its stylet into phloem cells, mesophyll tissues might form a resistance mechanism of cassava.

From a practical point of view, it must be emphasized that the addition of fertilizers as practised in Europe and North America, is very rarely done in traditional agriculture for cassava, because it is too expensive (Cock and Reyes, 1985). The use of nitrogen fertilizers in the NPK form at a rate of 60–75 kg N/ha. can only be considered if cassava is grown on an intensive, industrial scale. Nevertheless, agricultural methods such as burn-beating (burying partially burnt plant material into the soil), which constitutes a certain form of organic and mineral fertilization and which is widely done in some regions of Africa (Nzila, 1992), could be recommended to increase cassava tolerance. This is especially important since, in South Congo, the average yields of cassava crops managed by burn-beating are 4 tons greater than those of industrial crops, fertilized with NPK (urea = 150 kg/ha. or 67 kg N/ha.; triple superphosphate: 100 kg/ha. or 45 kg P_2O_5/ha.; potash: 150 kg K_2O/ha.; (Nzila, 1992). To confirm the present laboratory results, it will be necessary to compare burn-beating and NPK fertilization in a field trial; this trial will also have to be run in several places (one same variety may show varying degrees of resistance between one locality and another (Tingey and Singh, 1980) and should integrate the varietal component which influences the response of cassava to the addition of fertilizer (our study).

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