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Seasonal Changes of Secondary Compounds in the Phloem Sap of Cassava in Relation to Fertilisation and to Infestation by the Cassava Mealybug

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Abstract—The aim of the present study was to determine, by a field experiment, whether the seasonal variations in the levels of cyanide and rutin in the phloem, secondary compounds potentially implicated in cassava resistance to the cassava mealybug (*Phenaccocus manihoti*), could be influenced by different kinds of soil fertilisation (manure, NPK, KCl, lime, mulch). It was found that mean cyanide and rutin contents varied significantly with season, soil treatments and infestation. For each soil treatment, the highest contents of these compounds on uninfested plants was registered in the main dry season (Jul–Aug) for cyanide and at the end of the dry season (Jul–Aug) and beginning of the rainy season (Oct–Nov) for rutin. The infestation factor induced an increase in cyanide and rutin contents for 43 combinations of period and soil treatment among 48 in total. For cyanide content (phagostimulant function in mealybug), this increase appeared significant at the end of the rainy season (Apr–May) and in the main dry season (Jul–Aug) for control and KCl fertilisation. For rutin content (an unfavourable substance for the insect) significant increase was induced by infestation in each period for manure and mulch and especially in the main dry season (Jul-Aug) and at the beginning of the rainy season (Oct–Nov) for other soil treatments. Concerning the seasonal variations in the level of rutin, these results showed that mulch and manure are the best fertilisers to increase cassava resistance against mealybugs.

Key Words: *Phenacoccus manihoti*, Pseudococcidae, *Manihot esculenta*, cassava, antixenosis, antibiosis, phloem sap, nitrogen fertilisation, cyanogenic glucosides, plant phenolics, rutin

Résumé —L'objectif du travail présenté ici est de déterminer, par une étude de plein champ, si les variations saisonnières des teneurs phloémiennes en cyanure et rutine, composés secondaires potentiellement impliqués dans la résistance du manioc à la cochenille, pourraient être influencées par différentes fertilisations du sol (Fumier, NPK, KCl, Calcaire et Paillage). Les teneurs foliaires moyennes en cyanure et en rutine des plantes sont significativement modifiées par la saison, le traitement et l'infestation. Quelque soit le traitement, sur les plants non infestés, les teneurs maximales des deux composés sont mesurées pendant la grande saison sèche pour le cyanure et au début de la saison des pluies pour la rutine. D'une manière générale l'infestation par *P. manihoti* induit une augmentation des teneurs des deux composés en toute saison et quelque soit le traitement. En ce qui concerne le cyanure (substance ayant une fonction phagostimulante), la réponse à l'infestation est plus marquée en fin de saison des pluies (Av–Mai) et pendant la grande saison sèche (Jui-Aoû). En ce qui concerne la rutine (substance défavorable à l'insecte) on enregistre une forte réponse à l'infestation, en toute saison pour les traitements paillage et fumure organique. Pour les autres traitements, la réponse est plus marquée en saison sèche (Jui–Aoû) et au début de la saison des pluies (Oct–Nov). En terme de variation saisonnière des teneurs en rutine, nos résultats suggèrent que le paillage et la fumure organique améliorent la résistance du manioc à la cochenille.

Mots Clés: *Phenacoccus manihoti*, Pseudococcidae, *Manihot esculenta*, manioc, antixénose, antibiose, sève phloémienne sap, fertilisation azotée, glycosides cyanogénés, phénols, rutine

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Introduction

Since its accidental introduction into tropical Africa in the 1970s, the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), has been one of the major pests of cassava, *Manihot esculenta* Crantz. In 1982, a biological control programme was initiated by the successful introduction of the South American parasitoid *Apoanagyrus* (formerly *Epidinocarsis) lopezi* De Santis (Hymenoptera: Encyrtidae) (Herren, 1987). One possible strategy to enhance efficiency of the parasitoid would be the introduction of partially resistant varieties of cassava. For example, an increase in the developmental time of the pest on resistant cassava would lead to an extension of the access period for *A. lopezi*.

Following the identification of different categories of resistance in cassava (*sensu* Painter, 1951; Tertuliano et al., 1993) and the demonstration of an aphid-type phloem feeding behaviour of the cassava mealybug (Calatayud et al., 1994b), we showed that cyanogenic glucosides and rutin are translocated by the phloem sap of the cassava plant (Calatayud et al., 1994a). Cyanogenic glycosides (linamarin and lotaustralin) were shown to be implicated in the mechanisms of plant selection by the *P. manihoti* (antixenosis) and rutin was shown to inhibit its development (antibiosis) (Calatayud et al., 1994a).

Recently, under field conditions, we have shown that the important changes in the pest population observed throughout the seasons may be explained, in part, by the seasonal variations in the levels of the secondary compounds of the phloem. Rutin content decreased in the less resistant genotypes during the dry season, when stresses (infestation and drought) were prevalent, while the cyanide content (phagostimulant) of all tested genotypes increased in the dry season (Calatayud, 1993).

The synthesis of secondary compounds in the plant is more influenced by cultural practices (fertiliser, manure application and burn-beating) than by seasonal factors. In fact, the synthesis of secondary nitrogeneous compounds (alkaloids, cyanogenic glucosides and glucosinolates) increases with nitrate fertiliser treatment, while the synthesis of non nitrogenous compounds (phenolic compounds) increases when the plant is cultivated in a soil poor in nitrate fertiliser (Gershenzon, 1984; Waterman and Mole, 1989). The incidence of other factors influenced by fertilisation appears to be more variable: we have observed that a decrease in the phosphorous composition of soil induces an increase in the synthesis of cyanogenic glycosides, but it is not of alkaloids. The influence of different compound fertilisers on the biochemical characteristics of cassava has received little attention. Obigbesan and Fayemi (1976) observed an increase in the cyanide content of cassava cultivated using nitrate fertiliser.

This study sought to determine whether the seasonal changes of the secondary compound content of the phloem sap of cassava could be influenced by cultural practices.

Materials and Methods

Insects

Phenococcus manihoti reproduces by thelytokous parthenogenesis, and the clone we used was initially collected from cassava in a local garden in Brazzaville in 1985. Since then, a culture of *P. manihoti* has been maintained in the laboratory on cassava (*M. esculenta*, variety M'pembe) at 22–32°C and 12: 12 (L:D) photoperiod.

Plants

Our work was performed on MM79, a cassava genotype giving high yields in Congo (up to 25 tonnes/ha of roots per year) and characterised by its high resistance based upon both antixenosis and tolerance (Tertuliano et al., 1993; Le Rü and Tertuliano, 1993).

Field Study

Study site

The study was carried out in Brazzaville (Congo) from October 1990 to December 1991 on 700-m² piece of land that had been fallow for about 10 years. The main vegetation was *Chromolaena odorata* L. (Asteraceae). Its soil, representative of the regional soils of Brazzaville (Denis and De Champ, 1970) was typically ferralitic, strongly desaturated, of sablo-argilous texture at the surface and argilous below the surface, characterised by a pH of between 4.9 and 5.6 and low organic and mineral components. Chemical data of soil were provided by the laboratory of soil chemistry (ORSTOM-Brazzaville).

The monthly mean air temperature during the study period was 25.7°C (range 23.0–27.2) and the rainfall was 1653 mm with a dry season from June to September. A short dry season occurred in January and February.

Soil treatment

The five cultural practices recommended for cassava by Silvestre and Arraudeau (1983) were applied, namely KCl, mulch, manure, lime and NPK; the last four were known to influence the assimilation of nitrogen by plants (Soltner, 1985). The KCl, lime and NPK treatments were applied twice during our experiment: four days before taking cuttings and six months after, in accordance with Silvestre and Arraudeau (1983). Mulch and manure treatments were applied according to the results of field trials performed between 1986 and 1990 in different localities in south Congo, in order to determine the optimal growth conditions for cassava by Arrivetz (1990).

- control (no treatment)
- mulch treatment: 10 t/ha of dry weight of mowed turf of *Paspalum* sp.
- lime treatment: 1 t/ha of ground Madingou's calcareous at 52.7 % of CaO
- KCl treatment: 50 kg/ha of KCl fertiliser
- NPK treatment: 250 kg/ha of NPK 15/15/15 fertiliser
- manure treatment: 30 t/ha of bovine manure at 15% of nitrogen.

Our experiment involved 5 replicates of 6 soil treatments and 2 states (infested or uninfested), making a total of 60 experimental units. Each unit contained 9 plants of cassava of which 1 was in the centre of the unit and 8 at the edge in order to limit the effects of neighbouring soil treatments. Only the plant situated in the centre of the experimental unit was used for the infestation or as control. Fertilisation treatments were randomly attributed to each experimental unit in the field (i.e. 10 experimental units per treatment). Thirty-centimetre cuttings cassava genotype of MM79 were planted vertically on each unit in November 1990. The field was cultivated following a traditional method used in Congo (weeding at the end of the dry season and hoeing at the beginning of the rainy season). The upkeep of the plot involved two weedings and two hoeings during the 3rd and 6th months of culture, and an anti-mite treatment (Hexythiazox/Cesar) was applied each 3rd month.

Infestation with mealybugs

Four infestation periods were used: January–February (short dry season), April–May (end of the rainy season), July–August (main dry season) and October–November (beginning of the major rainy season). Thirty plants (5 per soil treatment) were artificially infested on the 3rd and 4th leaves under the apex with 200 neonate larvae (L1) from our laboratory colony of *P. manihoti*. The use of a thick smear of birdlime at the base of infested leave petioles prevents colonisation of the other parts of the plant by mealybugs. 30 other plants were left uninfested (control). The duration of the infestation was one month and all infested plants were examined twice a week. Mealybugs were added or removed when necessary to maintain a constant population. Between each infestation period, all the mealybugs were removed from the infested plants. We kept the control plants from being infested by examining them fortnightly and removing any mealybugs found.

After one month of infestation, the leaf areas were measured and the rutin and cyanide contents of dry leaf extracts were assessed in the infested and uninfested (control) leaves (2 leaves per plant for each of the 60 experimental units) corresponding to the same foliar level on the plant.

Phloem sap collections

Extracts were collected between 0800 and 1000 h. Because of difficulties in collecting phloem sap by stylectomy with mealybug, we used a centrifugation method modified from Rohringer et al. (1983). Three mature leaves per plant were cut without their petioles, washed in distilled water and wiped. They were then enveloped in a nylon muslin (0.05 mm) and centrifuged at 4000 g for 20 min. in a Sorvall SS 34 rotor at 0°C. All extracts were freeze-dried, weighed (± 0.1 mg) and stored at -20°C until required.

The fluids obtained by this method were the routine samples used for all comparative experiments in this study. Although containing exudates from phloem vessels (sugars are 80% sucrose), the samples are extensively contaminated with material from other tissues. However, the speed of centrifugation was selected to avoid breaking the cell walls.

Chemical analyses

Rutin. Extracts were dissolved in 250 µl of 50% methanol and centrifuged at 15,000 g for 15 min to remove solids. Twenty microlitres (20 µl) were injected on a C-18 RP-HPLC column with UV detection at 320 nm (Spherisorb S5ODS2, 4.5 x 250 mm, from Prolabo, FRA). Isocratic elution with water, acetonitrile and acetic acid (74.6, 23.4, 2% v/v) was performed at a flow rate of 0.8 ml/min. Rutin was used as the standard.

Cyanides. Dry samples were dissolved in 250 µl of distilled water and free cyanides were assayed by spectrocolorimetry with a commercial kit, using KCN as a standard (Spectroquant 14800, Merck, GER). CN values were supposed here to reflect total cyanogenic compounds in extracts, although cyanides are mainly in bound form in the plant.

The units used throughout this work (mg/g of dry weight), although not representing actual concentrations in the plant, are a good comparative index as they reflect the relative 'investment' in secondary chemistry as compared to the total solutes present in a sample. In some instances, they may be a better estimate than true concentration data, which may vary due to dilution effects due to natural physiological responses.

Assessment of water stress of cassava

Among the parameters dependent on drought stress of cassava, as defined by the Centro Internacional de Agricultura Tropical (CIAT) Cali, Colombia (Annual Reports), we used the leaf area, a decrease of which indicates a drought stress. The assessment of the foliar surface was calculated using the equation:

$$\mathrm{Log}_e(\mathrm{LA}) = -7.47 + 2.460\,\mathrm{Log}_e\,(\mathrm{MLL}) \qquad (1)$$

where LA= foliar area in cm^2; and MLL = length of the median lobe in cm (Hammer, 1980). This non-destructive method is easily applied in the field.

Statistical analysis

For analysis of variance, the three factors: fertilisation, infestation and season were considered as a fixed model. Area of leaves, rutin and cyanide contents were all found to be homogeneously distributed between factors, as tested with the Kolmogorov-Smirnov test (homoscedasticity hypothesis for ANOVA). For each significant factor ($P < 0.05$) the means were compared using Fisher's PLSD multiple range test. These statistics were completed using the Statview software (Abacus Concepts, USA).

Results

Results corresponding to assessment of the water stress condition of the plants under different fertilisation regimes, infestation status and season are given in Table 1. The 3-way ANOVA analysis (3 factors: soil treatment, season and infestation) showed that the foliar area varied significantly with the season and the infestation factors. The mean foliar area for uninfested plants was maximal in Jan–Feb (129.5, 150.9 and 168.0 cm^2 respectively) for manure, KCl and lime fertilisations, then decreased at the end of the rainy season (Apr–May), reaching a minimum value in the main dry season (19.1, 28.6 and 44.2 cm^2 respectively for manure, lime and mulch fertilisation). The greatest reduction of foliar area (85% decrease) due to drought stress was observed with the manure fertilisation. It increased significantly at the beginning of the main rainy season (Oct–Nov), though with values inferior to those in Jan–Feb.

For each soil treatment and for each period studied, the infestation factor induced a decrease of the mean foliar area. Nevertheless, this decrease was significant only for the main dry season (Jul–Aug) and for KCl and NPK fertilisations.

The mean rutin contents varied significantly with season, soil treatment and infestation, while the mean cyanide contents varied significantly with season and infestation (Tables 2 and 3). For each soil treatment, the highest contents of these compounds on uninfested plants were registered at the beginning of the rainy season for rutin (7.0 to 8.8 mg/g of dry weight), and during the main dry season for cyanide (0.9 to 1.6 mg/g of dry weight).

The infestation factor induced an increase in cyanide and rutin contents for each period and soil treatment. The increase in cyanide content appeared more significant at the end of the rainy season (Apr–May) for mulch, lime and KCl treatments and in the main dry season (Jul–Aug) for NPK, manure and control treatments. Cyanide content of uninfested cassava and the infested/uninfested ratio decreased significantly at the beginning of the rainy season (Oct–Nov). The

Table 1. Seasonal foliar areas of MM79 cassava variety, infested (in) and uninfested (ui) for different soil treatments (Control, lime, KCl, NPK, manure), (mean ± SE) and results of 3-way ANOVA (fertilisation, infestation and season factors)

		Periods			
Treatment	Infestation	Jan–Feb	Apr–May	Jul–Aug	Oct–Nov
Control	ui	131.2 ± 10.4 a.u	97.5 ± 11.2 a.v	43.1 ±16.7 a.w	71.0 ± 19.3 a.vw
	in	106.3 ± 23.3 a.uw	85.7 ± 13.1 a.uw	16.2 ± 7.3 a.v	54.9 ± 17.4 a.u
Mulch	ui	123.6 ± 9.1 a.u	118.0 ± 18.1 a.u	44.2 ± 8.3 a.v	116.1 ± 32.2 a.u
	in	116.3 ± 17.9 a.u	102.5 ± 15.8 a.u	30.4 ± 9.1 a.v	87.8 ± 27.8 a.u
Lime	ui	168.0 ± 9.5 a.u	113.5 ± 11.9 a.v	28.6 ±10.5 a.w	121.3 ± 15.2 a.v
	in	140.5 ± 12.4 a.u	100.7 ± 9.7 a.u	23.5 ± 3.8 a.v	131.2 ± 35.6 a.u
KCl	ui	150.9 ± 11.7 a.u	109.9 ± 12.0 a.v	40.2 ±11.9 a.w	109.8 ± 17.4 a.uv
	in	145.9 ± 6.0 a.u	103.8 ± 7.6 a.v	16.5 ± 5.8 b.w	70.1 ± 22.6 a.v
NPK	ui	132.1 ± 7.9 a.u	151.4 ± 9.2 a.u	28.8 ± 5.4 a.v	85.7 ± 26.7 w
	in	124.8 ± 15.3 a.u	125.3 ± 21.1 a.u	10.7 ± 5.7 b.v	77.3^{+}
Manure	ui	129.5 ± 14.9 a.u	112.2 ± 11.4 a.u	19.1 ± 4.2 v	101.2 ± 9.1 a.u
	in	111.3 ± 12.9 a.u	93.3 ± 10.2 a.uv	12.9	66.3 ±17.11 a.v

Factors of ANOVA: Period (A), 0.0001; Treatment (B), 0.0598; A x B, 0.1945; Infestation (C), 0.0101; A x C, 0.7948; B x C, 0.7139; A x B x C, 0.9971.
Means followed by the same letter are not different at 5% level (Fisher's PLSD test following ANOVA).
a,b: column comparison (infestation factor); u,v,w: line comparison (season factor).
$^{+}$Only one datum available.

Table 2. Seasonal mean contents of cyanide of phloem sap of MM79 cassava variety, infested (in) and uninfested (ui) for different soil treatments (Control, Lime, KCl, NPK, Manure), (mean ±SE) and results of 3-way ANOVA (fertilisation, infestation and season factors)

		Periods			
Treatment	Infestation	Jan–Feb	Apr–May	Jul–Aug	Oct–Nov
Control	ui	0.4 ± 0.1 a.u	0.6 ± 0.2 a.u	1.5 ± 0.4 a.v	0.7 ± 0.1 a.u
	in	0.4 ± 0.1 a.u	1.2 ± 0.3 b.v	3.7 ± 0.8 b.w	0.6 ± 0.1 a.u
Mulch	ui	0.3 ± 0.1 a.u	0.4 ± 0.1 a.u	1.1 ± 0.1 a.v	0.7 ± 0.2 a.u
	in	0.5 ± 0.2 a.u	0.8 ± 0.3 a.u	1.9 ± 0.8 a.u	1.0 ± 0.2 a.u
Lime	ui	0.5 ± 0.1 a.u	0.3 ± 0.1 a.u	1.6 ± 0.2 a.v	0.9 ± 0.2 a.w
	in	0.8 ± 0.3 a.u	0.7 ± 0.1 a.u	2.8 ± 0.8 b.v	0.9 ± 0.3 a.u
KCl	ui	0.6 ± 0.1 a.u	0.4 ± 0.1 a.u	0.9 ± 0.4 a.u	0.9 ± 0.3 a.u
	in	1.0 ± 0.2 b.u	1.5 ± 0.5 b.u	2.1 ± 0.6 b.v	0.8 ± 0.2 a.u
NPK	ui	0.5 ± 0.1 a.u	0.3 ± 0.1 a.u	1.3 ± 0.4 v	0.5 ± 0.2 u
	in	0.7 ± 0.2 a.u	0.5 ± 0.1 a.u	2.5^{+}	0.6^{+}
Manure	ui	1.0 ± 0.1 a.u	0.4 ± 0.1 a.u	1.5 ± 0.5 v	0.4 ± 0.1 a.u
	in	1.3 ± 0.2 a.u	0.7 ± 0.3 a.u	2.8^{+}	0.5 ± 0.1 a.u

Factors of ANOVA: Period (A), 0.0001; Treatment (B), 0.0643; A x B, 0.0694; Infestation (C), 0.0001; A x C, 0.0001; B x C, 0.14; A x B x C, 0.3062.
Means followed by the same letter are not different at 5% level (Fisher's PLSD test following ANOVA).
a,b: column comparison (infestation factor); u,v,w: line comparison (season factor).
$^{+}$Only one datum available.

increase in rutin content induced by mealybug infestation was observed for manure and mulch fertilisations for each period. However, it was observed that without fertiliser (control) mealybug infestation induced a significant increase in rutin content; the ratio of rutin content in leaves of infested to uninfested plants was 1.5:1 and 1.6:1 respectively in July–August and in October–November. This response created unfavourable conditions for the development of *P. manihoti*. Nevertheless, this response was inferior to those obtained for mulch and manure fertilisations. The corresponding ratios for each period were higher than 1.4. The highest ratio of rutin content in leaves of infested: uninfested plants, 1.98:1 was obtained for mulch fertilisation, at the beginning of the rainy season (Oct–Nov).

Table 3. Seasonal mean contents of rutin of phloem sap of MM79 cassava variety, infested (in) and uninfested (ui) for different soil treatments (Control, Lime, KCl, NPK, Manure), (mean ± SE) and results of 3-way ANOVA (fertilisation, infestation and season factors)

Treatment	Infestation	Periods: Jan–Feb	Apr–May	Jul–Aug	Oct–Nov
Control	ui	3.8 ± 0.3 au	2.9 ± 0.6 au	5.8 ± 1.2 av	6.9 ± 0.7 av
	in	4.5 ± 0.6 au	3.7 ± 0.6 au	8.7 ± 1.4 bv	11.4 ± 1.9 bv
Mulch	ui	3.7 ± 0.2 au	3.1 ± 0.2 au	6.7 ± 0.9 av	7.0 ± 0.4 av
	in	6.3 ± 1.1 bu	4.5 ± 0.4 bv	13.0 ± 1.7 bw	13.9 ± 1.9 bw
Lime	ui	4.4 ± 0.2 au	3.8 ± 0.2 au	6.8 ± 0.8 av	7.2 ± 1.0 av
	in	3.9 ± 0.6 au	4.8 ± 0.3 au	10.9 ± 2.1 bv	10.0 ± 1.8 bv
KCl	ui	3.9 ± 0.7 au	2.9 ± 0.2 au	7.6 ± 1.2 av	8.4 ± 0.5 av
	in	4.9 ± 1.2 au	4.4 ± 0.5 bu	11.9 ± 1.4 bv	9.2 ± 0.8 av
NPK	ui	4.4 ± 0.3 au	2.4 ± 0.2 av	8.4 ± 0.9 aw	8.8 ± 1.0 aw
	in	5.3 ± 0.7 au	4.2 ± 0.6 bu	11.2^{+}	11.9^{+}
Manure	ui	3.0 ± 0.3 au	2.5 ± 0.2 au	7.5 ± 0.8 av	7.4 ± 0.5 av
	in	5.2 ± 0.7 bu	4.0 ± 0.6 bu	13.5^{+}	13.8 ± 1.1 bv

Factors of ANOVA: Period (A), 0.0001; Treatment (B), 0.0191; A x B, 0.0757; Infestation (C), 0.0001; A x C, 0.0001; B x C, 0.1001; A x B x C, 0.2017.

Means followed by the same letter are not different at 5% level (Fisher's PLSD test following ANOVA). a,b: column comparison (infestation factor); u,v,w,x: line comparison (season factor).

$^{+}$Only one datum available.

Discussion and Conclusions

For each type of fertilisation, the observed decrease in leaf area during the dry season and its increase at the beginning of the rainy season showed that the plants were in drought stress in the dry season. El-Sharkawy and Cock (1987) and Yao et al. (1988) observed the same result on cassava, which they interpreted as being a mechanism to limit water loss from the plant. Seasonal leaf area variations were not affected significantly by mealybug infestation. Furthermore, our results indicate that the variations were independent on fertilisation and seemed to depend more on the tolerance of the cassava variety to drought.

For each soil treatment (except KCl fertilisation), the cyanide content increased in the dry season (Jul–Aug) and decreased significantly at the beginning of the rainy season (Oct–Nov). Previous work has suggested that cyanides are phagostimulant to the mealybug (Calatayud et al., 1994 a and b), so the increase of these compounds in cassava leaves promoted the fixation of *P. manihoti* on the plant. On the other hand, the significant decrease of these compounds at the beginning of the rainy season induced less favourable conditions for the insect. The infestation factor results showed that the KCl fertilisation induced a significant increase in cyanide content (as for control soil treatment) in the dry season, when the abundance of *P. manihoti* in the field was high. These results confirmed the hypothesis that KCl fertilisation was not sufficient to help protect the cassava against the mealybug.

Previous work has suggested that rutin is an unfavourable substance for insect development and that the defensive response of cassava to mealybug attack was realised by a significant increase in rutin content (Calatayud et al., 1994 a and b). In this context, our results showed that the mulch and manure fertilisation were the best fertilisers to help protect the plant against *P. manihoti*. In fact, for each period, these soil treatments induced a significant increase in rutin when plants were infested. This response was also observed for plants cultivated without fertilisation (control), but only at the beginning of the rainy season (Oct–Nov).

We observed that the seasonal changes of cyanide and rutin contents, as for leaf area, were not dependent on fertilisation. The lack of influence of different soil treatments on the development and the physiology of cassava in the dry season may result in a limited assimilation of the major nutrients of the soil (N, P, K and Ca) caused by drought stress (Gutierrez et al., 1988).

In many other models, the assimilation of nitrogen by the plant induces favourable conditions for mealybug populations (Thompson, 1941; Fennah, 1959; Salama et al., 1972; Smirnoff and Valero, 1975; McClure, 1980; Campbell, 1984; Washburn et al., 1987). These authors suggest a positive response of insects due to an increase in the nitrogenous nutrient content of the plant without mention of probable changes in the content of allelochemicals. The increase in nitrogenous nutrients occasioned by nitrogen fertilisation depends mainly on the plant genotype. Scriber (1984) reported that this increase was better for herbaceous plants than for conifers. In addition, the positive response of herbivorous insects to nitrogen fertilisation may reflect a shift in a plant's carbon-nutrient balance away from the production of phenolic carbon-based compounds to nitrogen-based compounds (Waring and Cobb, 1992). The absence of relationships between the antibiotic resistance of cassava and foliar amino acid content, described by Tertuliano and Le Rü (1992), and the probable metabolisation in *P. manihoti* of nitrogen of cyanide compounds, which may represent the major nitrogenous source of the phloem sap of cassava (Calatayud, 1993), suggests that the cassava mealybug is less sensitive than other insects to changes in free amino acid content in the plant. Furthermore, the drought stress in the dry season induced a significant increase in foliar cyanide content for each soil treatment. Waring and Cobb (1992) showed a great diversity of herbivorous insect responses to fertilisation and observed a good response of the pest on conifers cultivated on poor soil. The major observations made during the past 20 years on *P. manihoti* have shown that the natural abundance of the pest occurred in the dry season and that the damage was less when cassavas were cultivated on soil rich in organic elements (Nwanze et al., 1979; Matile-Ferrero, 1978; Neuenschwander et al., 1989; 1990). These observations suggest close similarities with conifers (perennial plants). Our results on the effect of manure and mulch fertilisations on the synthesis of rutin (a potentially unfavourable substance to mealybug development) in infested cassava for each period studied are consistent with those earlier observations.

Although our results need confirmation with more tests including yield assessments, we can recommend to growers the cultivation of cassava with mulch or manure fertilisation in order to limit the damage induced by *P. manihoti* in the dry season. This recommendation has already been suggested by previous observations, where damage induced by the pest was rarely observed on cassavas cultivated in forest regions. Nevertheless, our results are interesting because they suggest that an organic fertilisation (manure or mulch), as is found in forest regions, induces favourable conditions of the soil around the tubers and roots of cassava (humidity, development of rootlets and microorganisms, etc.) thus facilitating the assimilation of nutrients of the soil under drought conditions and helping to protect the cassava against the pest in the dry season.

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