

# Seasonal changes in secondary compounds in the phloem sap of cassava in relation to plant genotype and infestation by *Phenacoccus manihoti* (Homoptera: Pseudococcidae)

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## Abstract

A previous study on the population dynamics of the cassava mealybug, *Phenacoccus manihoti* (Matile-Ferrero), on cassava has shown that populations increase during the dry season. The aim of the present studies was to determine whether these important changes in the pest population observed throughout the seasons in the field in the Congo, could be correlated with seasonal variations in the level of secondary compounds in the phloem, implicated in cassava plant resistance to *P. manihoti*. Our results showed that cassava was in drought stress conditions during the dry season (foliar area decreased). The combination of infestation and dry stress factors was clearly visible during the dry season. In fact, the decrease in leaf water potential together with infestation by *P. manihoti* was clearly observed at the end of the rainy season and during the dry season. The levels of the secondary compounds, rutin (an unfavourable substance to *P. manihoti*) decreased in the less resistant genotype, and cyanide (a phagostimulant substance) increased in each genotype in the dry season. These results may partially explain the important population changes in *P. manihoti* during the seasons.

## 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) (Euphorbiaceae) (Matile-Ferrero, 1977; Nwanze, 1982). A study of the population dynamics on cassava has shown an increase every year during the dry season when numbers multiply within 7-10 weeks from less than 10 individuals per stem (number usually seen in the rainy season) to about 100. These dramatic changes in number were observed at the time of important changes in plant physiology (cessation of the development and rising of sap) in relation to drought stress during the dry season (Le Rü *et al.*, 1991). Furthermore, in some years in January-February during the short dry season we observed a small increase in the numbers of *P. manihoti* (Fabres, 1981), suggesting that abundance could

have some relationships with the trophic quality of the cassava plant.

Biochemical characteristics of the plant, induced by environmental stress, strongly influence (positively or negatively) the population dynamics of a pest (Rhoades, 1983; White, 1984; Mattson & Haack, 1987; Larsson, 1989; Waring & Cobb, 1992). Drought stress often produces an increase in plant nitrogenous compounds, such as amino acids, especially proline (Mattson & Haack, 1987). This increase may have a favourable influence on the population dynamics of phytophagous insects (McNeill & Southwood, 1978). Drought stress also influences the secondary metabolism of plants, i.e. an increase in cyanogenic glucoside and alkaloid content. For phenolic compounds, the conflicting results in the literature cannot be used to show any relationship between the drought stress and their levels in the plant (Gershenson, 1984; Waring & Cobb, 1992). The influence of drought stress on the biochemistry of the cassava plant has received little attention. De Bruijn (1973) observed an increase in the cyanide content of the cassava plant under drought stress conditions.

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In a previous work we have shown that *P. manihoti* is mainly phloemphagous (Calatayud *et al.*, in press), and that cyanogenic glycosides and rutin are translocated by the phloem sap of the cassava plant (Calatayud *et al.*, 1994). Cyanogenic glycosides (linamarin and lotaustralin) were shown to be implicated in the mechanisms of the plant selection by *P. manihoti* (antixenosis) and rutin was shown to inhibit development (antibiosis) (Calatayud, 1993).

It therefore seemed interesting to determine whether the important changes in the pest population, observed throughout the seasons during field studies in the Congo, could be correlated with seasonal variations in the levels of the secondary compounds of the phloem which are implicated in the resistance to *P. manihoti*. These secondary compounds vary according to genotype, and in the present study two genotypes with differing contents of these substances (Calatayud, 1993) and characterized by different levels of resistance to *P. manihoti* (Tertuliano *et al.*, in press) were investigated.

## Materials and methods

### Insects

*P. 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 L:D 12:12.

### Plants

Our work was carried out on two genotypes of cassava which had been previously characterized by different resistance levels based upon both antixenosis and antibiosis to *P. manihoti* and by different intrinsic rates of population increase ( $r_c$ , after Laughlin, 1965), which is a good way of estimating antibiosis. The cassava plants used were *M. esculenta* variety MM79 ( $r_c=0.155$ ) and the Faux caoutchouc hybrid of *M. esculenta* × *M. glaziovii* ( $r_c=0.141$ ) whose  $r_c$ s proved to be statistically different (Tertuliano *et al.*, in press). If we consider both antixenosis and antibiosis Faux-caoutchouc was preferred more than MM79 by *P. manihoti* in the field.

### Phloem sap collections

The following extracts were collected between 08.00 h and 10.00 h. Because of difficulties in collecting phloem sap by stylectomy of *P. manihoti*, we used a centrifugation method modified from Rohringer *et al.* (1983). Leaves were cut without their petiole, 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 Sorvall SS'34 rotor at 0°C. All extracts were freeze-dried, weighed ( $\pm 0.1$  mg) and stored at  $-20^\circ\text{C}$  until used.

The fluids obtained by this method were the routine samples used for all experiments in this work for comparative purposes. Although containing exudates from phloem vessels (sugars are 80% sucrose), these fluids are extensively contaminated by chemicals from other tissues. The speed of centrifugation was therefore selected to keep cell walls intact.

### Chemical analyses

Rutin: extracts were dissolved in 250  $\mu\text{l}$  of 50% methanol and centrifuged at 15,000 g for 15 min to remove solids. 20  $\mu\text{l}$  were injected on a C18 RP-HPLC column with UV detection at 320 nm (Spherisorb S50DS2, 4.5 × 250 mm, from Prolabo, FRA). Isocratic elution with a mobile phase of 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 standard.

Cyanides: dry samples were dissolved in 250  $\mu\text{l}$  of distilled water and free cyanides were assayed by spectrophotometry with a commercial kit, using KCN as 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.

### Assessment of water stress of cassava

Parameters dependent on drought stress of cassava, as defined by the International Center of Tropical Agronomy (CIAT) at Cali in Colombia (annual reports), are the leaf surface areas and leaf water potential. A decrease in either of these parameters indicates drought stress. The assessment of the leaf surface area was calculated using the equation:  $\text{Log}_e(\text{LA}) = -7.47 + 2.460 \text{Log}_e(\text{MLL})$  (LA: leaf area in  $\text{cm}^2$ ; MLL: length of the median lobe in cm) (Hammer, 1980). This non-destructive method is easily applied in the field.

The leaf water potential was measured between 10.00 h and 12.00 h using a pressure chamber (PMS Instrument 650). One leaf without its petiole was introduced into the chamber and nitrogen was injected until the pressure of the leaf was equilibrated with the pressure of the chamber. The leaf water potential is the pressure reading when the meniscus of the section of the petiole becomes convex. It is expressed in bars.

### Study in the field

The study was carried out in Brazzaville (Congo) from November 1990 to December 1991 on 700  $\text{m}^2$  of land that had been fallow for about ten years. The main vegetation was *Chromolaena odorata* (Compositae). The soil of the experimental plot represented the regional soils of Brazzaville (Denis & De Champ, 1970) and was typically ferralitic, strongly desaturated, of sablo-argilous texture at the surface and argilous at depth and characterized by a pH of 4.9–5.6. Data on the soil chemistry were provided by the Laboratory of Soil Chemistry (ORSTOM-Brazzaville). The organic and mineral components of the soil (data not shown) were balanced by a N/P/K (15/15/15) treatment prior to the experiment.

The monthly mean air temperature during the study was 25.7°C (23.0–27.2) and the rainfall was 1653 mm with a dry season from June to September. We also observed a short dry season during January and February (fig. 1). Climatic data were provided by the ORSTOM meteorological station at Brazzaville.

The 20 cm cuttings of the two genotypes studied were planted vertically in a random arrangement in the experimental plot in November 1990. The field was cultivated following a traditional method used in Congo (weeding at the end of the dry season and ploughing with a hoe at the

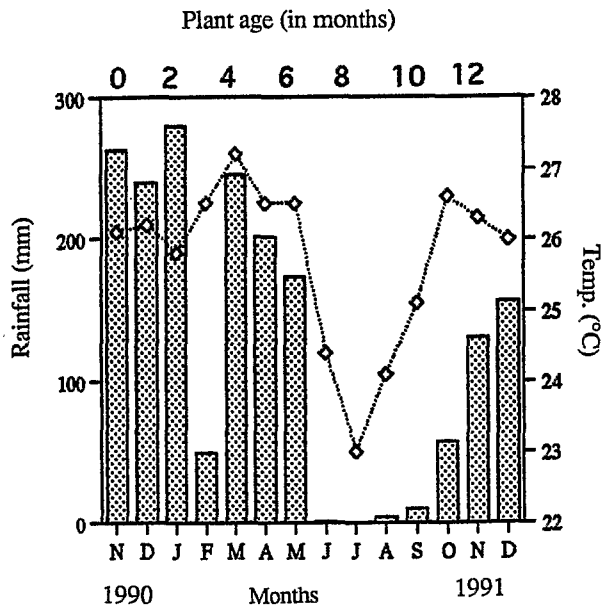


Fig. 1. Meteorological conditions (monthly rainfall and mean air temperature) recorded throughout the study period. Bar chart for mean rainfalls (in mm) and graph for mean temperatures (in °C). The plant age 0 corresponds to planting time in our field experiment.

beginning of the rainy season). The upkeep of the plot involved two weedings and two hoeings during the third and sixth months of culture, and an anti-mite treatment was carried out each third month to control mite populations.

*Infestation by mealybugs*

To study the population dynamics of *P. manihoti*, four infestation periods were used: January-February (short dry season, stated as Jan-Feb), April-May (end of the rainy season, stated as Apr-May), July-August (main dry season, stated as Jul-Aug) and October-November (beginning of the major rainy season, stated as Oct-Nov). On the day of infestation, for each genotype, we estimated the foliar area of the twentieth leaf under the apex (foliar level where the foliar area is more significant on the plant). Twenty plants were artificially infested on the second and third leaves below the apex with 200 neonate larvae (L1) from the laboratory culture of *P. manihoti* and 20 control plants remained uninfested. 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. For each infestation period we used only plants that had not been previously infested.

After one month of infestation, for each genotype the measure of leaf water potential and the analyses of rutin and cyanide of dry extracts were assessed in the infested and uninfested (control) leaves corresponding to the same foliar level on the plant.

*Statistical analysis*

For analysis of variance, factors 'genotype', 'infestation' and 'season' were considered as a fixed model. Leaf area, leaf water potentials and rutin and cyanide contents were found

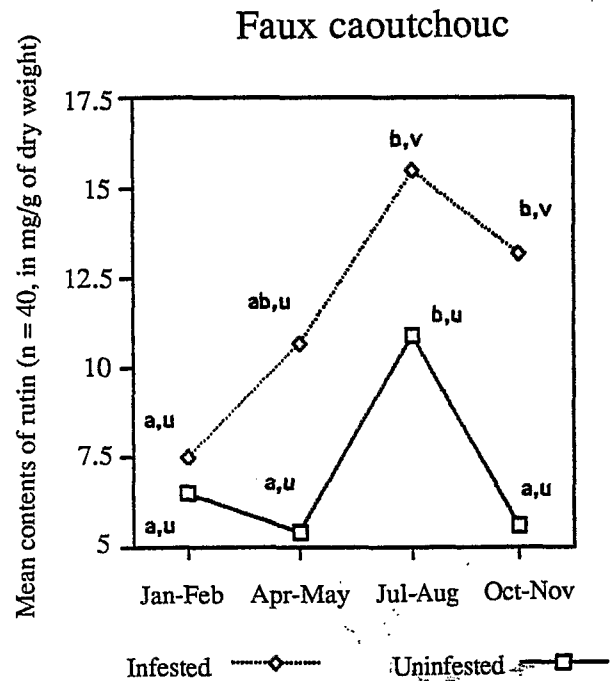
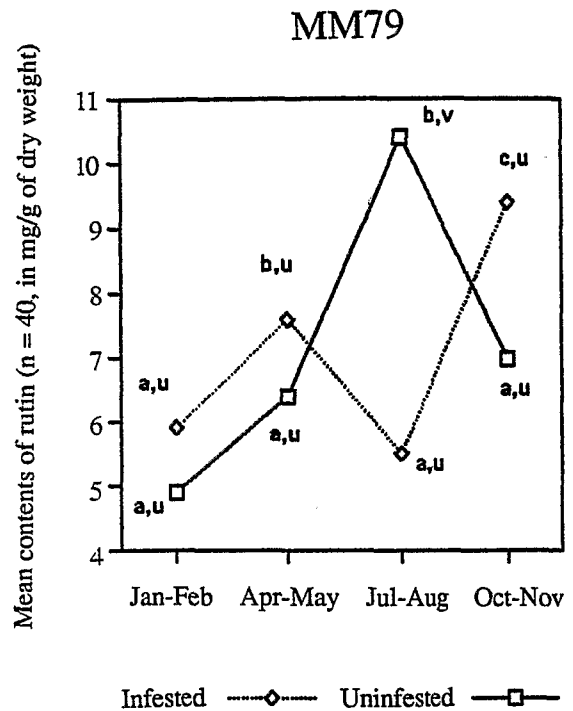


Fig. 2. Seasonal mean contents of rutin of phloem sap for two cassava genotypes, infested and uninfested. Results 3-way ANOVA (genotype, infestation and period factors): genotype (A): 0.0017; period (B): 0.0002; A×B: 0.1474; infestation (C): 0.0018; A×C: 0.0014; B×C: 0.0537; A×B×C: 0.1386. Means followed by the same letter are not different at 5% level (Fisher's PLSD test following ANOVA). a,b: period comparison; u,v: infestation comparison.

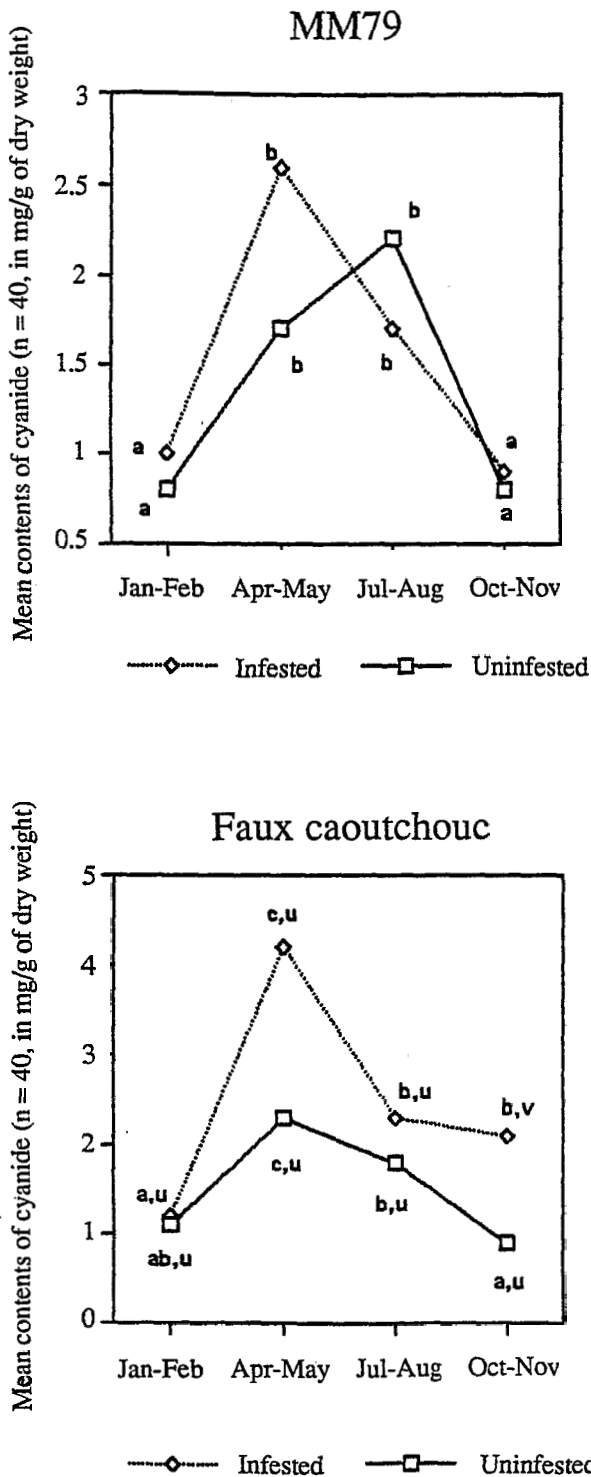


Fig. 3. Seasonal mean contents of cyanide of phloem sap for two cassava genotypes, infested and uninfested. Results 3-way ANOVA (genotype, infestation and period factors): genotype (A): 0.0077; period (B): 0.0001; A×B: 0.2383; infestation (C): 0.0068; A×C: 0.0582; B×C: 0.0505; A×B×C: 0.6242. Means followed by the same letter are not different at 5% level (Fisher's PLSD test following ANOVA). a,b: period comparison; u,v: infestation comparison.

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 (table 1 and figs 2 & 3). These statistics were completed using the Statview software (Abacus Concept, U.S.A.).

## Results

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 caused by artefacts or due to natural physiological responses.

*Water stress of plants (table 1).* For each genotype, the mean foliar area was maximal in Jan-Feb (204.0 and 404.0 cm<sup>2</sup>, respectively for MM79 and Faux caoutchouc), then decreased at the end of the rainy season (Apr.-May) reaching a minimal value in the main dry season (99.6 and 123.0 cm<sup>2</sup>, respectively for MM79 and Faux caoutchouc). It increased significantly at the beginning of the main rainy season (Oct-Nov) though with inferior values compared with the values in Jan-Feb.

The leaf water potential of control plants varied significantly with the season. For each genotype, a significant increase in this parameter value was observed between Jan-Feb (-6.2 and -5.2 bars, respectively for MM79 and Faux caoutchouc) and April-May (-3.9 and -3.5 bars, respectively, for MM79 and Faux caoutchouc), then a decreasing trend at the beginning of the rainy season in Oct-Nov. The leaf water potentials of infested plants were lower than for uninfested plants. This significant difference was observed at the end of the rainy season (April-May) and in the main dry season (July-Aug).

*Rutin and cyanide contents of phloem sap in uninfested plants (figs 2 & 3).* The highest values of rutin content were registered in the dry season (10.4 and 10.9 mg/g of dry weight, respectively, for MM79 and Faux caoutchouc in July-Aug). The highest values of cyanide contents were registered at the end of the rainy season (1.7 and 2.3 mg/g of dry weight in April-May for MM79 and Faux caoutchouc, respectively) and during the dry season (2.2 and 1.8 mg/g of dry weight in July-Aug for MM79 and Faux caoutchouc, respectively). On the other hand, the lowest values of cyanide contents were seen at the beginning of the rainy season (0.8 and 0.9 mg/g of dry weight in Oct-Nov for MM79 and Faux caoutchouc, respectively).

*Rutin and cyanide contents of phloem sap in infested plants (figs 2 & 3).* Seasonal changes of the rutin contents depended on the genotype. For MM79, the rutin content was lowest in the short and the main dry season (5.9 mg/g of dry weight in Jan-Feb and 5.5 mg/g of dry weight in July-Aug). For the hybrid Faux caoutchouc the mean content of rutin increased in the main dry season and at the beginning of the rainy season (15.5 mg/g of dry weight in July-Aug and 13.2 mg/g of dry weight in Oct-Nov). If infested vs uninfested plants were compared, the value of rutin content in infested plants

Table 1. Seasonal foliar areas and seasonal leaf water potentials for two cassava genotypes, infested and uninfested (mean  $\pm$  SE) and results of 2-way and 3-way ANOVA (genotype, infestation and period factors).

Genotypes Treatments	Foliar areas (in cm <sup>2</sup> , n=80)		Leaf water potentials (in bars, n=40)			
	MM79 uninfested	Faux caoutchouc uninfested	MM79 uninfested	MM79 infested	Faux caoutchouc uninfested	Faux caoutchouc infested
	Periods					
Jan-Feb	204.0 $\pm$ 9.8 c	404.3 $\pm$ 15.9 c	-6.2 $\pm$ 0.4 au	-4.7 $\pm$ 0.4 v	-5.2 $\pm$ 0.4 au	-5.9 $\pm$ 0.5 u
Apr-May	111.9 $\pm$ 7.1 a	174.8 $\pm$ 13.9 b	-3.9 $\pm$ 0.1 b,v	-5.1 $\pm$ 0.2 u	-3.5 $\pm$ 0.1 c,v	-4.8 $\pm$ 0.3 u
Jul-Aug	99.6 $\pm$ 5.4 a	123.0 $\pm$ 7.3 a	-3.8 $\pm$ 0.2 b,v	-5.3 $\pm$ 0.3 u	-4.3 $\pm$ 0.2 b,v	-5.7 $\pm$ 0.5 u
Oct-Nov	141.5 $\pm$ 10.2 b	141.0 $\pm$ 13.0 ab	-4.6 $\pm$ 0.3 bu	-5.4 $\pm$ 0.4 u	-4.6 $\pm$ 0.4 ab,u	-5.2 $\pm$ 0.5 u
Factors of ANOVA						
genotype (A)	0.0001		0.8924			
period (B)	0.0001		0.0001			
A $\times$ B	0.0001		0.4359			
infestation (C)			0.0001			
A $\times$ C			0.1353			
B $\times$ C			0.0008			
A $\times$ B $\times$ C			0.0459			

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

was significantly higher than in uninfested plants in the dry season (July-Aug) and this difference was even more apparent at the beginning of the rainy season (the infested/uninfested ratio was, respectively, 1.4 and 2.3). On the other hand, for MM79 we observed a significant decrease with infestation during the main dry season July-Aug (the same ratio was 0.5).

Similarly seasonal changes of cyanide content depended on the genotype. For MM79, the cyanide content was higher at the end of the rainy season (Apr-May) and in the main dry season July-Aug (2.6 and 1.7 mg/g of dry weight, respectively). For the hybrid Faux caoutchouc, the values were higher at the end of the rainy season Apr-May (4.2 mg/g of dry weight). For each genotype, as for the control plants, there was a significant decrease at the beginning of the rainy season (in Oct-Nov). If we compare infested vs uninfested plants, the values of cyanide contents were greater in infested plants of Faux caoutchouc in Oct-Nov (infested/uninfested ratio was 2.8). For both genotypes during the rest of the year the infestation remained without effect.

### Discussion and conclusion

The decrease in leaf area during the dry season and its increase at the beginning of the rainy season show that both genotypes are under drought stress in the dry season. El-Sharkawy & Cock (1987) found the same result on cassava and suggested that a decrease in leaf area was a way to limit water loss from the plant. In our study, this mechanism for saving water was observed after a significant increase in the leaf water potential (in control plants) at the end of the rainy season (April-May), which physiologically might be interpreted as the storage of free water in the plant before the dry season begins.

The decrease in the leaf water potential with infestation indicates that *P. manihoti* induces a stress at the end of the rainy season and during the main dry season in both genotypes. A similar decrease in leaf water potential has been reported by Riedell (1989) in wheat infested by *Diuraphis noxia* Mordvilko (Homoptera: Aphididae). This author has suggested that infestation induces a change in stomatal conductance which results in a decrease in the absorption of water by the plant. When both factors are combined (infestation and season) very little change in the leaf water potential is observed in infested plants throughout the year. Infestation causes further stress, masking seasonal variations which can be seen clearly in the control plants. The decrease in leaf water potential with infestation appears to be related to a decrease in the content of free water in the plant which effectively disrupts the primary and secondary metabolism of the plant. The increase in the rutin content in plants, following drought or pest stress, is clearly seen in our results. Moreover, the combination of these two stress factors reveals that response to stress varies according to plant genotype: rutin levels increase in Faux caoutchouc (susceptible genotype) and decrease in MM79 (resistant genotype) when stress is high. Similarly, the cyanide content increases in response to drought and pest stress. In the latter case, the increase is less obvious except in Faux caoutchouc (susceptible genotype) during the Oct-Nov period. It is interesting to note that, for each genotype, the levels of cyanide (secondary compound having a phagostimulant effect on the mealybug during plant selection) are linked

with less resistance during the dry season precisely when populations increase rapidly in the field, and, with more resistance at the beginning of the rainy season, when the populations decrease.

This study shows that both types of stress induce a similar response in the variations in the levels of secondary compounds. It also demonstrates that when the stress level is high (on infested plants during the dry season), the overall response decreases (Faux caoutchouc), or even reversed (MM79). This non-linear response to stress has been described previously in a study by Mattson & Haack (1987) on the levels of allelochemicals in plants which increased on moderately stressed plants and decreased on highly stressed plants. For the cassava variety MM79, the decrease in the levels of allelochemicals, induced by a high stress, results in a lower resistance of the plant to *P. manihoti* at the time when populations increase rapidly during the dry season.

The results show that the drought stress on cassava during the main dry season induces changes in the levels of secondary compounds involved in the resistance of the plant, which has a positive influence on the population dynamics of *P. manihoti*. The effect of drought stress on insect densities has been studied extensively. In Homoptera, Miles *et al.* (1982) have observed that the development of *Brevicoryne brassicae* Linnaeus (Aphididae) was best on stressed cabbage and Dorschner *et al.* (1986) have found that wheat under drought stress, and infested with *Schizaphis graminum* Rondani (Aphididae), suffered more damage than control wheat. To our knowledge, very few studies have examined the effect of drought stress on secondary metabolism of plants. Waring & Cobb (1992) reported seven studies until 1991, but none related to Homoptera. These authors have reported a great diversity in the response of phytophagous insects to drought stress and have observed that the hypothesis of a positive effect on phytophagous insects (White, 1984; Rhoades, 1979 & 1985) is only valid for conifers; in herbaceous plants, stress usually has a negative effect on phytophagous insects. Our results suggest that the response of cassava to drought stress is similar to that in conifers. However, it should be emphasized that the development of a plant is strongly linked to seasonal variations, and consequently it is difficult to dissociate the effects of seasonal variations and development on secondary metabolism. This is true for the levels of phenolic compounds, the most abundant allelochemicals in cassava, which are particularly sensitive to seasonal variations (Waterman & Mole, 1989). The relative abundance of phenolic compounds found in cassava is consistent with the suggestion made by the last authors, i.e. that plants, adapted to poor edaphic conditions and strong light intensity (natural conditions of cassava crops), synthesize more carbon-based secondary compounds. Although, the metabolic pathways of these compounds are well known today, the regulatory mechanisms remain unclear (Waterman & Mole, 1989).

To conclude, this study has shown that, under experimental conditions in the field, seasonal variations of the levels of secondary compounds in cassava can partly explain the abundance of *P. manihoti* during the dry season. The great genotypic variability of the response of cassava plants to drought stress, in terms of secondary compounds, indicates the need for further investigations, testing other genotypes which have a greater resistance to the mealybug. It would also be useful to carry out a similar field study

under different ecological and edaphic conditions, in particular in a forest region and on soil which is richer in organic material and where the numbers of *P. manihoti* are reduced (Neuenschwander *et al.*, 1989), or indeed where they are rarely observed (Matile-Ferrero, 1978; Le Rû, personal observation).

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