

## HOST SELECTION BEHAVIOUR BY THE CASSAVA MEALYBUG *PHENACOCCLUS MANIHOTI* MATILE-FERRERO (HOMOPTERA, PSEUDOCOCCIDAE): POTENTIAL INFLUENCE OF BIOCHEMICAL COMPOUNDS OF THE LEAF SURFACE

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### ABSTRACT

In the context of integrated control against the cassava mealybug *P. manihoti*, the aspect of varietal resistance and particularly the antixenotic component of the resistance was studied. Three varieties of cassava (*Manihot esculenta* Crantz, Euphorbiaceae) which are known to have different degrees of antixenotic resistance, and a weed of cassava's fields (*Talinum triangulare* Jack, Portulacaceae) were considered. The study of host selection behaviour and the sensorial equipment of the mealybug led us to the conclusion that the plant surface can provide informations which influence the choice of the insect. Physical and chemical features of the phylloplane, which can play a role in that choice, were then analysed. The observation of the surface of the three cassava varieties shown no particular physical structures (glands or pegs). However, the study of biochemical compounds of the leaf surface and their potential role on the choice of the insect reveals behavioural differences between leaves washed or not with methanol. The preliminary H.P.L.C. analyses of methanol extracts revealed the presence of two groups of compounds and one isolated compound, which was suspected to be linamarine, a cyanogenic glycoside of cassava.

**KEY WORDS** : mealybug, behaviour, plant surface biochemistry

### INTRODUCTION

The cassava mealybug *P. manihoti*, native of South America, was accidentally introduced in the seventies in Tropical Africa (Silvestre, 1973; Herren, 1987) and is now responsible for considerable damages in the cassava fields where the losses can reach 30 % of the yield. For the last ten years, control methods have been investigated to set mealybugs's populations under the economic threshold. In the context of biological control, *Epidinocarsis lopezi* De Santis (Hymenoptera, Encyrtidae), native of South America, was introduced in 1982 in Africa with success. However, this parasitoid is not efficient in the poor soil cultivated areas (Neuenschwander *et al.*, 1990; Le Rü *et al.*, 1991). The actual studies are oriented to the utilisation of integrated control in order to improve the ability of *E. lopezi* to maintain low populations of the mealybug. It is in that context that our researches on the varietal aspect of the resistance are developed.

According to Painter (1951), resistance can be defined into three components: the antixenosis, the antibiosis and the tolerance. Our main interest goes to the antixenosis which is concerned with the understanding of plant selection mechanisms by the insects. According to Bernays & Chapman (1994) it is then essential to analyse the behaviour and note its variations in relation with the resistance degree of the plant. These authors gave a good summary of the steps affecting the plant research by the insect. First, the localization is influenced by physical (colour, shape...) and chemical (odour) characteristics of the plant. Then, when it is on the plant surface, the insect is again confronted to a choice which can depend of the physical (texture) and chemical characteristics (taste, odour) of the plant surface which determine the plant acceptance. The researches of Stadler (1986) have demonstrated that the insect behaviour is influenced by those stimuli.

In the Hemiptera, the acceptance behaviour was often examined and shown similitudes among species (Bernays, 1988). In aphids (Homoptera, Sternorrhyncha), which are alike in nutritional aspect to the mealybugs, the acceptance is a succession of steps: the testing of the plant surface and of external tissues, the penetration and examination of the phloem (Klingauf, 1987). The testing is a succession of walks and probes in which the insect moves its antenns, drums the plant surface with its legs, its labium and its antennae according to a typical pattern (Ibbotson & Kennedy, 1959). Only the cuticle or the epidermis are usually explored by the labium and the stylets. Klingauf (1987) consider that the rejection of a host plant occurs at this moment. The penetration of the stylets into the plant is generally unidirectional and it is followed by the examination of the suitability of the phloem sieve.

At the time of our early researches, the description, for the first time for a mealybug, of the testing behaviour of the plant surface led us to a new classification of the four investigated plants on the basis of antixenosis. This kind of approach was important because the small number of works devoted to this subject shows that phytophagous insects "exhibit behavior patterns that can be interpreted as an examination of the surface quality and acceptance or rejection may follow without further testing" (Chapman & Bernays, 1989).

The objectives of the present work is to proceed our research by observing the testing behaviour of mealybugs on leaves of which the characteristics of the surface were modified by washing with various solutions. The results would allow us to underline chemical compounds present at the leaf surface and to be able to influence the acceptance of the plant.

**MATERIAL AND METHODS**

All the observations were realized in the laboratory of Gembloux, in a room at 26 ± 2°C and 70-75 % H.R.

**Plants**

The study was realized on three varieties of cassava *M. esculenta* Crantz (Incoza, M'Pembé and Faux-caoutchouc), the last one being a hybrid of *M. esculenta* and *M. glaziovii* Muel. Arg.. A weed of cassava fields, *Talinum triangulare* Jack (Portulacaceae), on which mealybugs settle sometimes, was also studied. Those varieties were choosen on the basis of their early description in term of antixenosis (Chapman *et al.*, 1993): it decreases from Talinum to Incoza, from Incoza to M'Pembé and from M'Pembé to Faux-caoutchouc. Plants were obtained from cuttings of twenty centimeters, planted vertically at 2/3 of their height in soil embedded in plastic bags (30 x 22). They were put in a glasshouse during the 9-10 leaf stage and after that in an room at 26 ± 2 °C, 70-75 % H.R. and a photoperiod of 17h.

**Insect**

The mealybug's strain comes from Brazzaville. It was maintained in the laboratory of Gembloux in a room at 26°C ± 2°C and 75-80 % H.R.

**Selection of the fixation site**

The experiment was conducted with first instar mealybugs which are responsible for the colonization of the plant. The day before the experimentation, mature eggs (hatching within a period of time of 24 H) are put in the obscurity in the room. In the morning, when the insects are particularly active, one hour before the observation, they are replaced in the light to assure to obtain larvae of the same age. One larva is put on the upper face of a young leaf and its behaviour is described during 90 minutes according to Klingauf's model on aphids (1969-1973). Side changes, number of probes, time spend on each side are noted. A total of 20 mealybugs were observed for each variety. The results are represented on an ethogram.

**Selection of the fixation site after deeping leaves in water or methanol**

In what concerned water washing of the phylloplane, the leaf is immersed for 10 minutes in ultrapure water before depositing the mealybug on the upper side. The observations begin when the leaf is fully dry and the items aforementioned are taken into account. Ten mealybugs are studied. Ethograms are again established.

In the case of methanol, only one part of the leaf (lobe) is examined. It is immersed for 5 secondes in 15 ml of methanol (analytical grade). The lobe is fixed with the lower face up on a leaf blade to avoid that the leaf margins become curled. It is surrounded by a honey ring which limits attempts to escape. Washing time is brief to decrease the risk of extraction within the leaf. A new series of controls had to be done because of the modification of the experimental device. It consists of the observation of the behaviour of 10 mealybugs on a lobe without any washing. Each of these two experimentations gave rise to 10 ethograms.

**RESULTS AND DISCUSSION**

**Selection of the fixation site**

The results were represented by 9 ethograms called behavioural type 1 to type 9 (figure 1) described in table 1. Each variety is characterized by a different proportion of them.

A distinction between short term fixation (less than 15 minutes) and long term fixation (more than 15 minutes) is made. Indeed, this period allows the mealybug's stylets to pass through the epidermis (first barrier at the penetration) and to progress in the parenchymia cells.

The examination of the ethograms shows some trends among varieties:

- **Incoza** is mainly characterized by the type 1. It corresponds to mealybugs which move quickly to the lower leaf face (2,7 min) where after a while they make a long term probe (11,9 min). That kind of behaviour is similar to the one described by Klingauf (1969-1973) when an insect meet a host plant: it passes rapidly on the lower leaf face and stops walking.
- On **M'Pembé**, the selection of the fixation site is of type 4. The mealybugs prefer the upper leaf face where they make relatively rapidly a probe (20,2 min). However, 20 % of the individuals (type 5) exhibit instability by leaving the leaf and walking towards the stem. In conclusion, the behaviour observed is that of an insect on a host plant. Nevertheless it is less favorable than for Incoza.

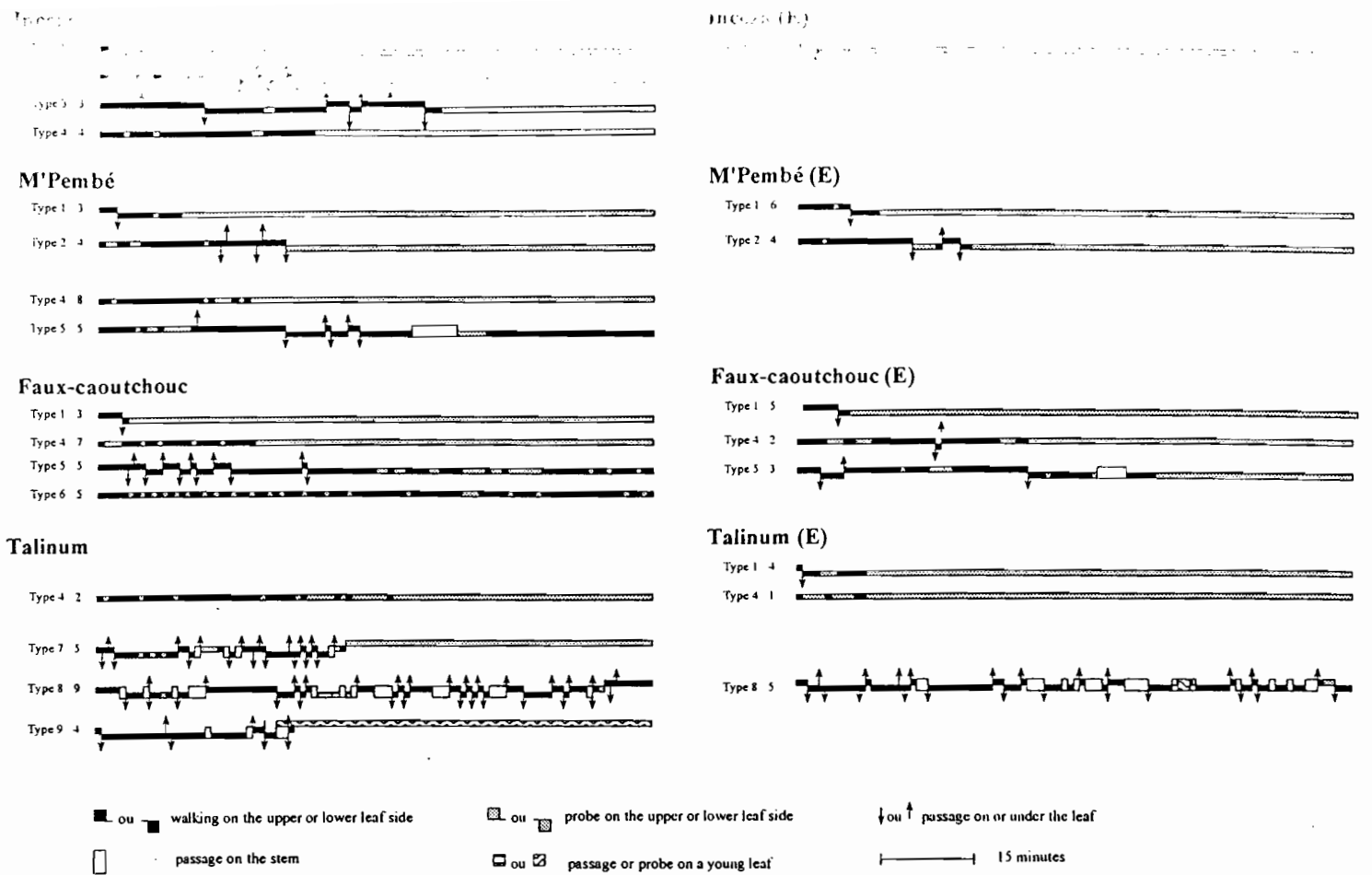


Figure 1: Characteristic ethograms of the mealybug testing behaviour observed during 90 minutes on leaves of the four plants washed (E) or not with water.

Type	Variety	n	tps (min)	tfs (min)	dd (min)	probes	side.ch	Particularities
1	Inco	9	2,7 ± 0,4	11,9 ± 1,9	9,8 ± 1,5	1 ± 0,3	1,7 ± 0,5	The mealybugs stay especially on the lower leaf side
	M'P	3	4,7 ± 2,9	12,3 ± 4,1	11,7 ± 3,7	1 ± 0,5	1,7 ± 0,7	
	Fc	3	6 ± 3,3	8,8 ± 4,6	8,8 ± 4,6	0	1 ± 0	
	Inco (E)	10	1,9 ± 0,6	6,8 ± 1,8	6,4 ± 1,7	0,4 ± 0,2	1 ± 0	
	M'P (E)	6	2,3 ± 1,2	10,5 ± 2,7	9,5 ± 2,5	0,8 ± 0,3	1 ± 0	
	Fc (E)	5	3,4 ± 1,1	12,5 ± 5,8	10,8 ± 4,9	0,8 ± 0,6	1,4 ± 0,4	
	Tali (E)	4	1,2 ± 0,4	12,7 ± 3,3	13,7 ± 5,7	1,5 ± 0,9	1 ± 0	
2	Inco	4	21,9 ± 5,5	35,6 ± 3,3	31,4 ± 3,7	4,5 ± 1,3	4 ± 1,3	Similar to the type 1 but increase of the time before a long term probe, of the time spent walking and of the number of side changes
	M'P	4	16,7 ± 4,7	27,5 ± 4,3	24,7 ± 4,6	1,7 ± 0,5	3,5 ± 0,9	
	M'P (E)	4	7,7 ± 3,8	31,2 ± 9,4	29,7 ± 9,2	1 ± 0,6	3 ± 1,4	
3	Inco	3	17,7 ± 5,4	70 ± 4,5	63,2 ± 3,9	4,3 ± 1,8	7,3 ± 2,6	Similar to the type 2 but accentuation of its characteristics
4	Inco	4	/	34,7 ± 6,9	36,9 ± 9,8	4,5 ± 1,5	0	No walk on the lower side was observed. The fixation occur always at the upper face and for more than 15 minutes
	M'P	8	/	20,2 ± 6,3	50,9 ± 6,3	2,2 ± 0,7	0	
	Fc	7	/	27,2 ± 4,2	32,6 ± 4,2	4,1 ± 0,5	0	
	Tali	2	/	48,5 ± 14	29,2 ± 18	5,5 ± 3,5	0	
	Fc (E)	2	/	21,5 ± 18,5	18,2 ± 15,2	1,5 ± 1,5	1 ± 1	
	Tali (E)	1	/	8,5	4	2	0	
5	M'P	5	29,8 ± 6,3	*66,5 ± 2,5	72,8 ± 5,5	*6,5 ± 1,5	5,4 ± 0,7	Probes occur indifferently at the upper of lower leaf side
	Fc	5	22,8 ± 5,9	*52,4 ± 1,7	68,3 ± 8,6	*4,5 ± 0,5	6,8 ± 1,6	
	Fc (E)	3	2,7 ± 0,8	59,3 ± 2,2	45,8 ± 6,8	4 ± 1,1	2,3 ± 0,3	
6	Fc	5	/	**58,5 ± 0	68,8 ± 7,3	**12 ± 0	0	Probes are short and occur always at the upper side. Lobe changes are observed
7	Tali	5	1,5 ± 0,8	42,1 ± 9,3	29,8 ± 5,6	5 ± 1,2	8,2 ± 2,4	Long term probe occur always at the upper side
8	Tali	9	11,2 ± 3,4	/	76,2 ± 3,0	/	15,2 ± 1,8	Attempts to escape are numerous (passing on the stem).
	Tali (E)	5	6 ± 4,3	**68	78,2 ± 6,1	**2	13,4 ± 3	No long term probes were observed
9	Tali	4	0,7 ± 0,2	33,6 ± 7,0	32,5 ± 7,8	0,7 ± 0,5	7 ± 3,6	Long term probes occurs on a young leaf

Table 1: Characteristics of the behavioural types detected during the observations (90 minutes) of 10 and 20 first instar mealybugs respectively on whole leaves washed or not with water (see table 2 for the legend)

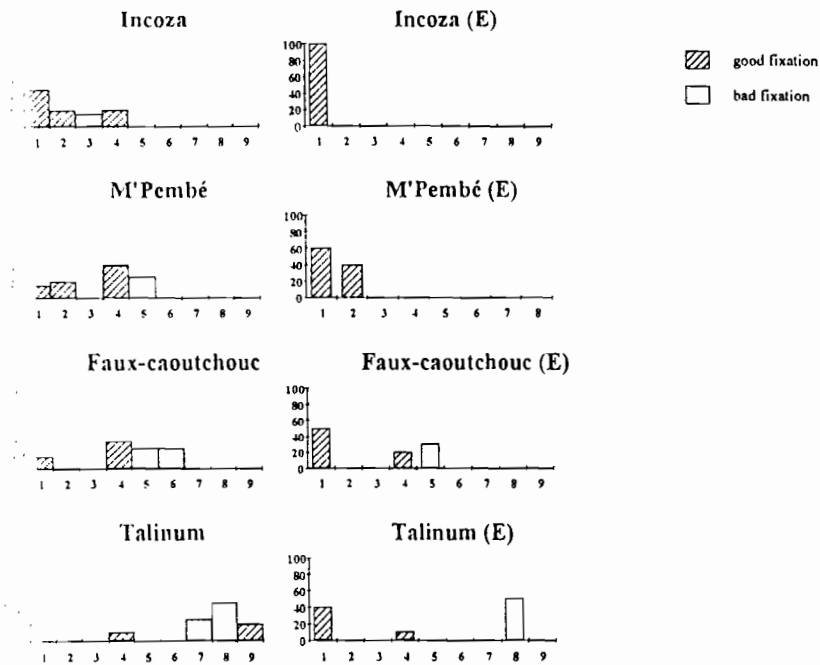


Figure 2: Characteristic histograms of the behavioural types (1 to 9) observed on leaves washed (E) or not with water

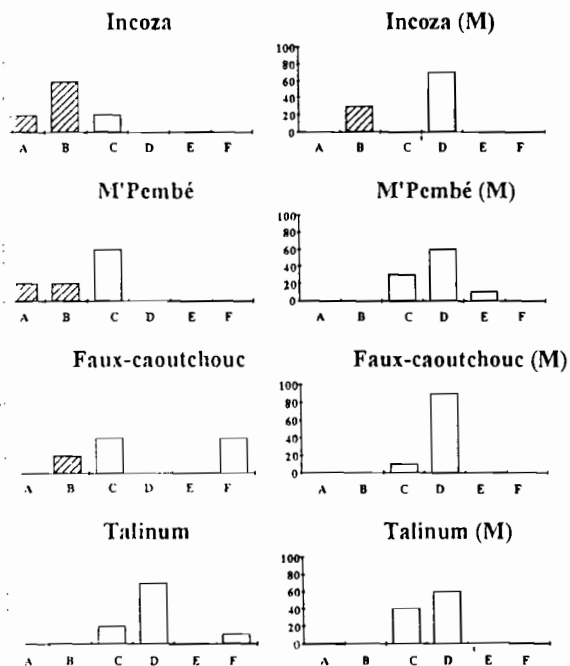


Figure 3: Characteristic histograms of the behavioural types (A to F) observed on leaf parts washed (M) or not with methanol

- On the **Faux-caoutchouc**, the behaviour is shared between types 4, 5 and 6. 60 % of the mealybugs stayed on the upper leaf face, 35 % of them made a long term probe and the remainder made numerous and short probes. 25 % percent of the mealybugs moved for long time (68,3 min.) changing of leaf face frequently (6,8). Such a behaviour is considered by Klingauf (1969-1973) to be that of an insect on a non host plant on which the time spend in walking is particularly on the upper leaf side.
- The **Talinum** is represented by the type 8. The mealybugs spend all the time walking (76,2 min), on either of the leaf faces and they go on the stem to escape (6,2). The non host character observed on Faux-caoutchouc is accentuated on the Talinum. Indeed, long time walkings are associated with attempts to escape.

In conclusion, this study has led to a new classification of the plants on the basis of antixenosis: it decreases from Talinum to Faux-caoutchouc, from Faux-caoutchouc to M'Pembé and from M'Pembé to Incoza.

### Selection of the fixation site after washing leaves with water

Five of the nine ethograms types encountered previously were established (types 1, 2, 3, 4 and 8) (figure 1). They were compared with those of the control experiment for each variety (figures 2 and 3).

- For **Incoza**, no behavioural differences were observed. The type 1 was again the most frequent (100 % of the individuals).
- In the case of **M'Pembé**, the fixation was successful too but this time, the mealybugs preferred the lower leaf face. The type 1 was observed at a frequency of 60 %.
- On the **Faux-caoutchouc**, a certain instability is again observed. The type 1 (50 %) reveals a good fixation on the plant while the types 5 (30 %) and especially the type 4 (20 %) are meaningful of a bad fixation on the plant (e.g. attempts to escape).
- About the **Talinum**, the behaviour was similar to that of the control. Type 8 (50 %), characterized by long time walking mainly at the upper leaf face, numerous face's changing and attempt to escape, was predominant.

The conclusion is that the fixation behaviour is as a whole the same after water extraction of leaves. The antixenosis improves from Incoza to M'Pembé, from M'Pembé to Faux-caoutchouc and from Faux-caoutchouc to Talinum.

### Selection of the fixation site after washing the leaves with methanol

The new series of 10 controls gives rise to four new types of ethograms (types A, B, C and F) (figure 4) described in table 2. Their analysis leads to the following characterization:

- On **Incoza**, the selection of the fixation site was in particular of type B (60 %). The mealybugs made relatively quickly a long term probe on the lower side of the lobe and they go rarely to the honey. Thus, even if the experimental device (procedure) had disturbed the mealybugs (need more time to made a long term probe), their behaviour was typical of that observed on a host plant.
- On the variety **M'Pembé**, while 40 % of the individuals made a long term probe on the lower leaf face, the remaining 60 % did not stop walking during 90 min, making some short term probes. Such a behaviour can be associated with that observed on a host plant but less favorable than Incoza.
- The **Faux-caoutchouc** is characterized by types C and F. The mealybugs made short term probes or walked during 90 min. The Faux-caoutchouc correspond to a non host plant.
- On the **Talinum**, the selection behaviour of the fixation site is especially represented by the type D. Most of the mealybugs walked all the time and went through the honey to quit the experimental

before 90 minutes were reached. It is evident that the Talinum is not appreciated by the mealybugs.

In conclusion, the mealybugs behaviour observed here led to the same classification as previously on the basis of the antixenosis. It increases from Incoza to M'Pembé, from M'Pembé to Faux-caoutchouc and from Faux-caoutchouc to Talinum.

The mealybugs behaviour on the control "lobes" was compared to that of the methanol washed lobes (figure 6):

- On the **Incoza**, contrary to the control which was typically represented by the type B, the washed "lobes" were characterized by the type D (70 %). So, only 30 % of the mealybugs made relatively quickly a long term probe while the remaining 70 % quit the experimented area before reaching 90 minutes. It is clear that the plant is no more suitable for the mealybugs.
- On the variety **M'Pembé**, we observed a similar evolution of the behaviour. 60 % of the individuals made a long term probe. The others made a long term probe or walked all the time while making some short term probes.
- The unsuitability of the **Faux-caoutchouc** is more accentuated in comparison with that of the control (generalization of type D).
- On the **Talinum** the same tendency can be observed with a good representation of type D (60 %) together with type C (40 %). This latter is characterized by the absence of long term fixation and by short walking periods.

From these conclusions can be drawn from these observations. First, the classification on the basis of the antixenosis is still the same than those of the controls, while the Faux-caoutchouc and the Talinum can be placed on the same range. Second, the washing with methanol have brought changes in the mealybugs behaviour. A diminution of the plant recognition is noted. So the methanol extracts need to be further investigated.

## CONCLUSIONS

The search of the chemical compounds which are susceptible to influence the mealybug in the choice of the plant by washing techniques gave interesting results. The first step (deeping the leaf in water while observing the mealybug) shows no behavioural differences when compared to intact leaves. On the other hand, the leaf washing with methanol have led to a diminution of the plant recognition in all the varieties.

At this stage of our knowledges, several hypothesis can be drawn:

- First, the methanol extraction is not limited to the surface of the leaf and is responsible of the destruction of enzymatic systems which deters the mealybugs. The spectroscopic samples analysis in the visible at 600 nm did not show any chlorophyll (Fiala *et al.*, 1990), suggesting that the destruction of chlorophylls in the plant is negligible.
- Second, there are residues of methanol on the leaf which disturb the mealybug. To test that hypothesis, the behaviour of mealybugs on filter paper with or without methanol washing was observed. No behavioural differences were noted as a proof of the absence of any trace of methanol.

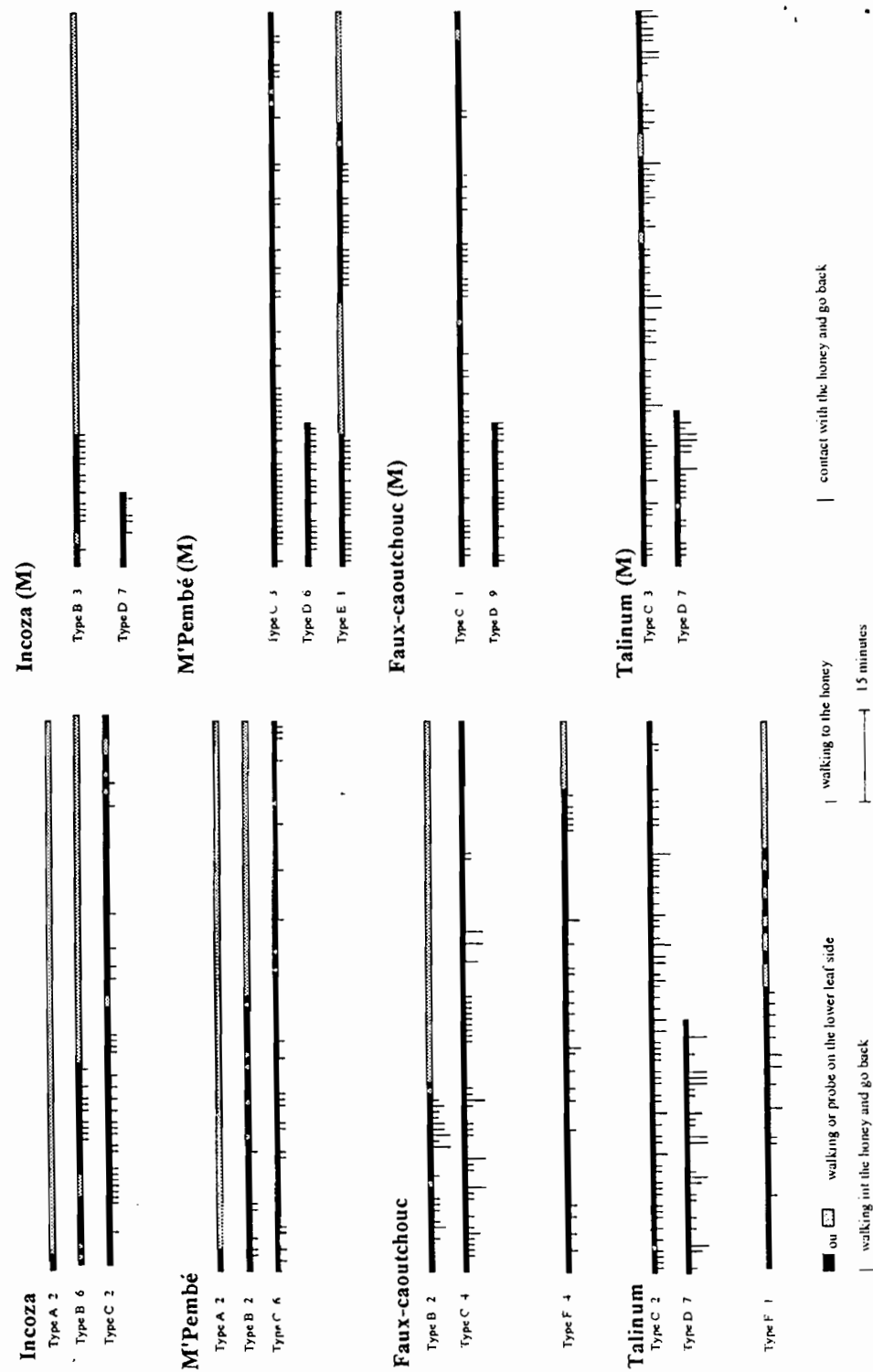


Figure 4 Characteristic ethograms of the mealybug testing behaviour observed during 90 minutes on leave parts (lobes) of the four plants washed (M) or not with methanol.

Type	variety	n	I.F.S.	MI	probes	particularities
A	Inco	2	7,5 ± 6	7 ± 5,5	1 ± 1	The mealybugs didn't try to go across the honing
	M'P	2	3,2 ± 1,7	3,2 ± 1,7	0	
B	Inco	6	41,7 ± 3,2	37,2 ± 3,3	1,2 ± 0,5	Similar to the type A but increase of the time before a long term probe, of the time spent walking and some contacts with th honing are observed
	M'P	2	48,7 ± 9,2	46,4 ± 10,5	3 ± 2	
	Fc	2	34,7 ± 0,2	33 ± 0	2 ± 0	
	Inco (M)	3	48,3 ± 8,3	47 ± 8,5	0,7 ± 0,3	
C	Inco	2	/	87,2 ± 2,2	/	The mealybugs walk all the time, making some short term probes Numerous attempts to escape are recenssed
	M'P	6	/	87,5 ± 0,9	/	
	Fc	4	/	89,7 ± 2,2	/	
	Tali	2	/	86,7 ± 2,2	/	
	M'P (M)	3	/	86,2 ± 1,8	/	
	Fc (M)	1	/	90	/	
	Tali (M)	4	/	86,6 ± 0,9	/	
D	Tali	7	/	43,9 ± 8,5	/	All the mealybugs go across the honing and leave the lobe before the end of the observation period
	Inco (M)	7	/	45,1 ± 6,4	/	
	M'P (M)	6	/	29,6 ± 9,4	/	
	Fc (M)	9	/	22,9 ± 4,5	/	
	Tali (M)	6	/	43,2 ± 8	/	
E	M'P (M)	1	24,5	51,5	2	The individuals show a certain instability on the plant (alternance of probes and walks)
	Fc	4	(* )74 ± 1	76,1 ± 4	*0	The mealybugs spend all the time in walking but each of them made a probe after 80 minutes
Tali	1	74,5	57	5		

Table 2: Characteristics of the behavioural types detected during the observations of 10 first instar mealybugs on parts of the leaves (lobes) during 90 minutes lenght without and with methanol wash.

Inco = Incoza, M'P = M'Pembé, Fc = Faux-caoutchouc, Tali = Talinum, (E) or (M) = washed with water or methanol, n: number of individuals, Tps : mean time after which insects passe on the lower leaf side, Tfs : mean time to make long term probe, wathver the leaf side, side.ch : mean number of side change on a period of 90 minutes, TFS: mean time to make a long term probe, dd: mean lenght of the total walk, probes: mean number of probes done before making a long term probe, /: no event was observed, \* or \*\*: only two or one mealybug(s) on 5 made a long term probe, (\*): only 2 mealybugs on 4 made a long term probe. All the means are completed by standard errors.

7. third, the methanol extraction have probably removed one or many feeding stimulant compound(s) which are present at the plant surface, on / in the waxes. It is clear that a little part of waxes is removed during the extraction procedure. The waxes are known to be often responsible for the choice of the insect for feeding and oviposition (Chapman & Bernays, 1989). Klingauf *et al.* (1971) show that the pea aphid, *Acyrtosiphon pisum* increases the probing duration in response to alkane extracted from the leaf surface of the broad bean *Vicia fabae*. About the beetles, Adati & Matsuda (1993) suggested that the insects can perceive both functional groups and the chain length of wax molecules with their sensory organs.

The confirmation of the third hypothesis is in progress and would be very essential for the research of resistant characters of cassava to the mealybug. The preliminary chemical analysis of the methanol extracts with H.P.L.C. showed notably one compound suspected as linamarine, one of the principal cyanogenic glycosides of cassava. A careful identification of this molecule is mandatory. Some analytical investigations are in progress.

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