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Electrically recorded feeding behaviour of cassava mealybug on host and non-host plants

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Abstract

The feeding behaviour of cassava mealybug (*Phenacoccus manihoti*) was analysed by the electrical penetration graph technique (EPG, DC-system) and by light microscopy. This study confirmed a typical phloem-feeding behaviour with an aphid-like predominance of extracellular pathways of stylets. Similarities of EPGs from mealybugs with those of aphids and whiteflies allowed adoption of standard pattern labelling. The main differences with aphid EPGs consisted of fewer but longer intracellular punctures (pd duration of 20 s *vs* 5–7 s for aphids), longer times to the first phloem-ingestion period and a lower motility of stylets within the phloem searching process. Comparison of the feeding behaviour on 6 plant types, including two occasional hosts (*Talinum* and poinsettia), one cassava hybrid and three common cassava cultivars (*Manihot esculenta*), allowed differentiation of penetration profiles. Intracellular punctures were shorter on occasional hosts, on which phloem finding and subsequent ingestion were markedly delayed although a great variability within plants persisted. Chemical analysis of some secondary plant substances revealed that none of the plants tested contained detectable amounts of alkaloids, and that cyanides were restricted to true hosts (cassavas and *Manihot* hybrid). Levels of total flavonoids did not differ between hosts and non-hosts, in contrast to those of phenolic acids. Preliminary correlations with EPG patterns are discussed.

Résumé

Le comportement alimentaire de la cochenille du manioc (*Phenacoccus manihoti*) a été étudié par électrographie de pénétration (EPG, analyse en courant continu) et par microscopie optique. Cette étude a permis de confirmer le caractère typiquement phloémophage de l'alimentation de cet Homoptère, présentant une large prédominance du trajet extracellulaire des stylets, comme cela est également observé chez les pucerons. Les similitudes des tracés EPG effectués sur cochenille et sur pucerons ou aleurodes ont permis d'adopter la même typologie du signal. Les principales différences avec les pucerons concernent le nombre moins important et la durée plus longue des ponctions intracellulaires (20 s en moyenne contre 5 à 7 s chez les pucerons), l'allongement du temps d'accès au phloème et une moins grande mobilité des stylets lors de la phase de recherche du phloème. La comparaison du comportement alimentaire sur 6 plantes, parmi lesquelles deux hôtes occasionnels (*Talinum* et poinsettia), un hybride et trois variétés courantes de manioc (*Manihot esculenta*), a permis de différencier plusieurs profils de pénétration. Les ponctions intracellulaires se sont révélées plus courtes sur hôtes occasionnels, qui induisent également, malgré la variabilité observée, un allongement net de la période de localisation du phloème et donc un retard à l'ingestion. Aucune des plantes testées ne contient d'alcaloïdes, et les composés cyanés se restreignent au genre *Manihot*, hôte naturel de la cochenille. Les flavonoïdes totaux ne permettent pas de distinguer plantes hôtes et non-hôtes, à la différence des acides phénoliques.

Introduction

Cassava is a tuber crop native to South America, introduced into Africa in the 1600s by the Portuguese. Today, it represents an important food staple for nearly 200 million people. The cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero, Homoptera: Pseudococcidae) is an oligophagous insect living mainly from cassava (*Manihot esculenta* Crantz, Euphorbiaceae) (Matile-Ferrero, 1976). During the early 1970s, *P. manihoti* was accidentally introduced into Africa, resulting in loss of cassava tuber yields and reduced quality of cutting material (Herren, 1981), because of severe growth disturbance. Since 1982, biological control has been initiated by successful introduction of the exotic south American parasitoid *Epidinocarsis lopezi* De Santis (Hymenoptera: Encyrtidae) (Herren, 1987). One of the possible strategies to enhance the efficacy of the parasitoid would be the introduction of non-favourable (i.e. 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 *E. lopezi* (van Emden & Wearing, 1965; Starks *et al.*, 1972; Dreyer & Campbell, 1987; Auclair, 1988).

Host plant resistance (HPR) is also by itself a pest control tool, especially useful in developing countries where chemical control is expensive and difficult to achieve on food crops. The 'antixenotic' resistance (Painter, 1958, Kogan & Ortman, 1978) to the cassava mealybug has previously been evaluated in Congo on different cassava cultivars (Tertuliano *et al.*, 1993). HPR genetics is central to breeding programs, but an understanding of the underlying biological mechanisms of resistance or tolerance is essential when dealing with questions such as durability, resistance pyramiding, pest variability or the need for biological or chemical markers of resistance. The purpose of this study, carried out in Congo, was to understand the details of the feeding behaviour of *P. manihoti* in relation to the selection of its host-plant. The behaviour of mealybugs has already been described in Coccidae (Pesson, 1944; Albrigo & Brooks, 1977) and in Pseudococcidae (Campbell, 1990; Molyneux *et al.*, 1990). All of these authors reported a phloemophagous behaviour, and our study was performed to identify the different leaf tissues that might play a role in host selection by *P. manihoti*. An assessment of the pathways followed by stylets through leaf tissue was obtained using electrical penetration graphs (EPGs) and light microscopy. For this first study of a coccid by EPG, the existing background work on aphids (Tjallingii, 1988) was used as a reference for a correlation of EPG patterns with various components of the stylet penetration process.

It is well documented that secondary compounds, in addition to nutritional factors, may have important functions in plant resistance to pests (Fraenkel, 1969; Kogan, 1977; Pickett *et al.*, 1992). Numerous data on aphids have illustrated the effects of alkaloids (Dreyer *et al.*, 1985; Smith, 1966; Wink & Witte, 1991), phenolic acids and flavonoids (Dreyer & Jones, 1981; Leszczynski *et al.*, 1985; Mc Foy & Dabrowski, 1984; Todd *et al.*, 1971) or cyanide compounds (Dreyer & Jones, 1981; Schoonhoven & Derksen-Koppers, 1976). In the present study, we investigated the possible links between the feeding behaviour of *P. manihoti* and the occurrence of these different classes of secondary compounds in the range of plants tested.

Materials and methods

Plants. We used six plant types differing in their susceptibility towards the cassava mealybug: Three cassava varieties (Incoza, Zanaga, 30M7; *M. esculenta* Crantz, Euphorbiaceae), the hybrid 'Faux caoutchouc' (*M. esculenta* × *M. glaziovii* Mull. Arg., Euphorbiaceae) and two other species, the poinsettia (*Euphorbia pulcherrina* Wild., Euphorbiaceae) and the *Talinum* (*Talinum triangularae* Jack., Portulacaceae). In natural conditions in Congo, *P. manihoti* lives mainly on cassava and on 'Faux caoutchouc', and severe population outbreaks occur on cassavas in the dry season (June–October). Nevertheless, some limited infestations were locally observed on talinum (Neuenschwander *et al.*, 1986), and under laboratory conditions, the insect can be reared on poinsettia (Boussienguet, 1984). We therefore used the two latter species as occasional hosts and called them substitute plants. The degree of susceptibility of the various plants utilised was previously evaluated by a choice test in the field (Tertuliano *et al.*, 1993, using the term 'degree of antixenosis'). This test was based on a visual estimation with ranking on a density scale: 0 for no infestation, 1 for a very low to low infestation (1 to 25 mealybugs per plant), 2 for a low to medium infestation (26 to 50), 3 for an average to high infestation (51 to 75) and 4 for a high to very high infestation (>75). According to this experiment, plants were ranked as follows: degree 0 was assigned to the more resistant plants (talinum and poinsettia),

while degrees 1/2/3/4 were assigned to cassava varieties Incoza/ Zanaga/ 30M7/ Faux Caoutchouc.

Ten-week-old plants were used for all experiments, grown in 30 cm pots in a shady environment. For light microscopy and standard EPGs on cassava, we used the M'pembe variety as a reference because it is the main variety grown in the Pool area in Congo, and it is very commonly infested with *P. manihoti* (susceptibility index 4). We did not include this variety in our comparative study because it was used for mass rearing of all experimental insects, thus bearing a special host status.

Insects. A culture of *P. manihoti* was maintained in the laboratory on cassava (*M. esculenta*, variety: M'pembe) at 22-32° C and L12:D12 photoperiod. Two other pseudococcids were used in some EPG analyses:

- *Ferrisia virgata* Cock. (Hom. Pseudococcidae), collected and reared on cassava.
- *Rastrococcus invadens* Williams (Hom. Pseudococcidae), reared on mango tree *Mangifera indica.*

Light microscopy. After a few days of infestation with adult mealybugs, infested tops of M'pembe plants were fixed in Halmi fixative (Gabe, 1968), rehydrated and embedded in paraffin. Cross sections were cut at 6 μm on a LKB microtome, dehydrated, stained with safranine-methyl blue (Locquin & Langeron, 1978) and viewed with a Leitz Dialux 20 light microscope.

Electrical monitoring. Stylet penetration was monitored by a single channel DC-EPG system (Tjallingii, 1988), with a 10^9 ohm input resistance (Model 'EPG 86' summer course, Van de Pers Inc., The Netherlands). Mealybugs were placed in a plastic petri dish for 1–2 min. A gold wire (2–3 cm, Ø25 μm) was then fixed on the dorsum of the insect with a water-based silver paint. The insect was connected to the amplifier before being placed on the upper side of a leaf, and experiments were carried out in a Faraday cage, at a temperature of 21–32 °C.

For reference EPGs (Figs. 2 and 3, Table 2), we used the cassava variety M'pembe and a data acquisition system consisting of a PC-based Kontron HPLC device (MT450 software and digitising card) converting and storing the (−5,+5V) EPG signal at a 100 Hz sampling rate. The stored signal was then transferred to a MacIntosh-based EPG analysis software (MacStylet) allowing off-line analysis and statistical treatment of the recordings (Rahbé *et al.*, 1993). More than twenty individuals were analysed to yield the standard mealybug parameters (Table 2).

For comparative EPGs on different host-plants, we used a paper chart recorder. Six different plant types were analysed in this experiment (Table 3). Three to six mealybugs per plant type were monitored on a standard EPG chart recorder (0–75 Hz, Mini-writer, Ankersmit, GER). Time 0 corresponded to the start of penetration by the insect. For each EPG recording, we performed comparative measurements either on the first 10 h of penetration, or until a long ingestion period (more than 4 h in phloem) was reached. Fourteen behavioural events were measured according to Tjallingii (1990) for each EPG recording and are described in Table 1.

In all EPGs, different electrical waveform 'patterns' can be distinguished. They were characterised by their duration, voltage level, relative amplitude, and electrical origin (Janssen *et al.*, 1989). Although the voltage level of a pattern may fluctuate and can be changed by adjusting the supplied voltage, an intra- and extracellular voltage level can always be distinguished, due to the transmembrane potential of the plant cell (Tjallingii, 1985b). The electrical origin indicates whether a pattern element is due to resistance fluctuations (R), electromotive forces (emf), or both (Tjallingii, 1985b). Apart from the above mentioned characteristics, peak and wave-shaped fluctuations could be distinguished in some patterns and are indicated here as 'peaks' and 'waves' respectively. Pattern labelling followed the aphid standards (Table 2), and sub-patterns were labelled 1, 2 ... when successive (type E1, E2) and I, II ... when alternate or relatively independent (type CI, CII, CIII ...), as already done for whitefly EPGs (Janssen *et al.*, 1989).

Plant extracts. 'Phloem sap': The day before collection, plant foliage was washed thoroughly; in the early morning of the next day (22–30 °C, L12:D12) the natural exudate dropping from the petiole of the top second and third leaves was collected in a capillary tube. Cassava natural exudates collected in this way are thought to be phloem secretions from companion cells, as no structural nectaries were detected at the exudation sites (Pereira & Splittstoesser, 1987).

'Extracellular fluids': A mixture of phloem sap and extracellular fluids of leaves were sampled by a centrifugation method modified from Rohringer *et al.* (1983). The top second and third leaves were cut without their petiole, washed in distilled water and wiped. They were then enveloped in a nylon muslin (0.05 mm)

Table 1. EPG parameters used to compare mealybug penetration behaviour on 6 plants. pd1 and pd2 refer to sub-patterns as defined in Fig. 2b. In text and Table 3, parameters from the 10 first hours of EPGs have an additional indication '−10' (e.g.: d-C-10 = total duration of pathway activity (C) during the first ten hours of recording)

Abbrev.	Parameter description	Units
t-Ei	time to sustained (>10 min) phloem ingestion	hours
t-E	time to first phloem puncture	hours
n-E	number of (short*) phloem punctures	#
d-E	total duration of (short*) phloem punctures	hours
ds-pd	(mean) duration of separate cell puncture (pd)	seconds
ds-pd1	(mean) duration of separate part (1) of pd	seconds
ds-pd2	(mean) duration of separate part (2) of pd	seconds
d-C	total duration of pathway activity (C)	hours
n-np	number of non-penetration (np) intervals	#
d-np	total duration of non-penetration	hours
n-pen(E)	number of penetrations with (short*) phloem puncture (E)	#
n-pen(xE)	number of penetrations without phloem puncture	#
d-pen(E)	duration of penetrations with (short*) phloem puncture	hours
d-pen(xE)	duration of penetrations without phloem puncture	hours

* the last phloem puncture (long, >4 h), on which the recording ended, was excluded from these parameters (see Materials and methods)

Table 2. Characteristics of cassava mealybug EPG patterns and comparison with aphid stylet activities

Pattern		Characteristics				Correlation with stylet activities in aphid
subpattern		Voltage level	Electrical	Frequency	Duration	(from Tjallingii, 1990)
periodic element		(extra/intracellular)	origin	(Hz)	(s/h)	
A		extra	nd (R?)		var.(≈2-10 s)	establishment of electrical contact
C:		extra		—	var.(->h)	extracellular pathway activities (incl. salivary sheat secretion)
CI		"			2-5 s	
	peak	"	nd	1-3		
	wave	"	nd	8-10		
CII		"	nd	8-11	var.	
CIII		extra+ 0*	nd (R?*)	—	var.(≈1-25 s)	
pd:		intra		—	17-23 s	short intracellular punctures during pathway (5-7s in aphids)
pd1		"			10-14 s	
	peak	"	emf	1-2		
	wave	"		13-15		
pd2		"	R*	9-12	5-14 s	
E:		intra		—		
E1		"	nd		var. (≈30-75 s)	E1 = stylet puncture of a sieve element
	peak			1-2		
	wave			10-12		
E2I		"			var.(->h)	E2= sustained ingestion from a sieve element
	peak		emf	1-5		
	wave		R	8-11		
E2II		"	nd (R?*)	5-6	var.	

* = see text for comments, nd = not determined

and centrifuged at 10 000 rpm for 20 min in a Sorvall SS 34 rotor.

All extracts were freeze-dried, weighed (± 0.1 mg) and stored at −20° C until used.

Chemical analyses. Alkaloids: extracts were resolubilised in 100 μl of distilled water and deposited on a TLC plate of Silicagel 60, developed in pure methanol, and visualised with Dragendorff reagent (Bounias, 1983). Caffeine and quinine were used as standards. Phenolics: Extracts were resolubilised in 250 μl of 50% methanol and centrifuged at 15 000 g for 5 min to remove solids. 20 μl were injected on a HPLC device with UV detection at 320 nm, a C18 column (Spherisorb S5ODS2, 4.6 × 250 mm, from Prolabo, FRA), and a mobile phase of aqueous acetonitrile (23.4% ACN, 2% acetic acid) at 0.8 ml/min. Data integration and identification of peak class (flavonoids/phenolic acids) by comparisons with standard phenolics allowed a quantification of the two classes of compounds. Phenolic acids were compared as p-coumaric acid equivalents and flavonoids as rutin equivalents (these two compounds have been identified in our cassava extracts). Cyanides: Extracts were resolubilised in 250 μl of distilled water, and cyanides assayed colorimetrically with a commercial kit (Spectroquant 14800, Merck, GER). KCN was used for calibration.

Statistical analysis. A Kruskal-Wallis test was used to analyse the EPG results. These results were also normalised ($\sqrt{X+1}$ transform) and subjected to Fisher's PLSD multiple range test. All these statistics were achieved using the Statview software (Abacus Concept, USA).

Results and discussion

Light microscopy. LM plates show that the stylets may go between cells (Fig. 1a) and through intercellular air spaces (Fig. 1b). Although in a number of situations the plates suggest intracellular positions of the stylet path (Fig. 1c), the large thickness of sections with respect to cell dimensions and the low resolution of LM do not allow any definitive conclusion. Most stylets seem to end in the vascular bundle (Fig. 1d), presumably in the phloem tissue, but whether near or inside a cell and the cell type involved remains unclear. Counting of stylet endings showed that out of 18 clear stylet figures, 14 penetrations reached a phloem element, the remaining 4 showing only mesophyll endings. Safranin appears to stain many side branches (Fig. 1c), of which only one contains the actual stylets. The stained material must be a secretion left in the empty branches, most likely the salivary sheath material. These indications fit very well with features of other phloem feeding homopterans.

Basic EPG wave-forms. Mealybug EPGs showed strong similarity with wave-forms produced by aphids, as described earlier (Tjallingii, 1978; Kimmins & Tjallingii, 1985; Tjallingii, 1985b, 1987, 1988, 1990). Therefore, we decided to label them similarly (Fig. 2 and 3, Table 2).

The first pattern at the start of penetration is typically of high amplitude, oscillating between the 0 V level and the highest values reached in the recording. It lasts a few seconds in general before sloping down to the more regular pattern C, sometimes after a precocious and generally atypical potential drop (Fig. 2a). It is similar to pattern A of aphids, representing the establishment of electrical contact between the mouthparts and plant tissues. Pattern B of aphids, indicative of the first salivary blobs secreted, are absent or at least much less visible in recordings from *P. manihoti.*

Pattern C comes rapidly afterwards (Fig. 2). It is a rather complex wave-form that was often maintained for considerable periods. Periods with high amplitudes alternated with periods of low amplitude. As in aphids, this alternation itself seemed to occur rather regularly at intervals of about 10 s, i.e. about 0.1 Hz (Fig. 2b, CI/CII). The other signal frequencies showed variations around 1–3 Hz (CI peaks), whereas smaller pieces ranged up to 8–10 Hz (CI and CII waves). Apparently, from these frequency analyses, the CI pattern could come from the intrusion of the 1–3 Hz peaks into a background CII pattern, but this is still hypothetical. Analogous to aphids, pattern C is likely to be related to stylet path activities, mainly stylet piercing and salivary sheath formation. Its occurrence for a long period, from labial contact with the epidermis onwards, suggested that penetration of the epidermis and the mesophyll, presumably up to the vascular bundle, is probably included in pattern C. This is also suggested by the LM plates (Fig. 1). No indications were found so far, however, for any signal details to be related to partial stylet withdrawal before making a new side branch (Fig. 1c). Sub-pattern CIII is a rather intriguing event occurring very rarely in certain recordings and more frequently in others. Characteristic is the occurrence of repeated high amplitude shifts towards an almost 0 V level (Fig. 2d). As it was inherent to pattern C, and occurred frequently before potential drops suggesting intracellular punctures, it cannot be interpreted as ruptures in electrical contacts (such as in pattern A). Although no electrical adjustments have

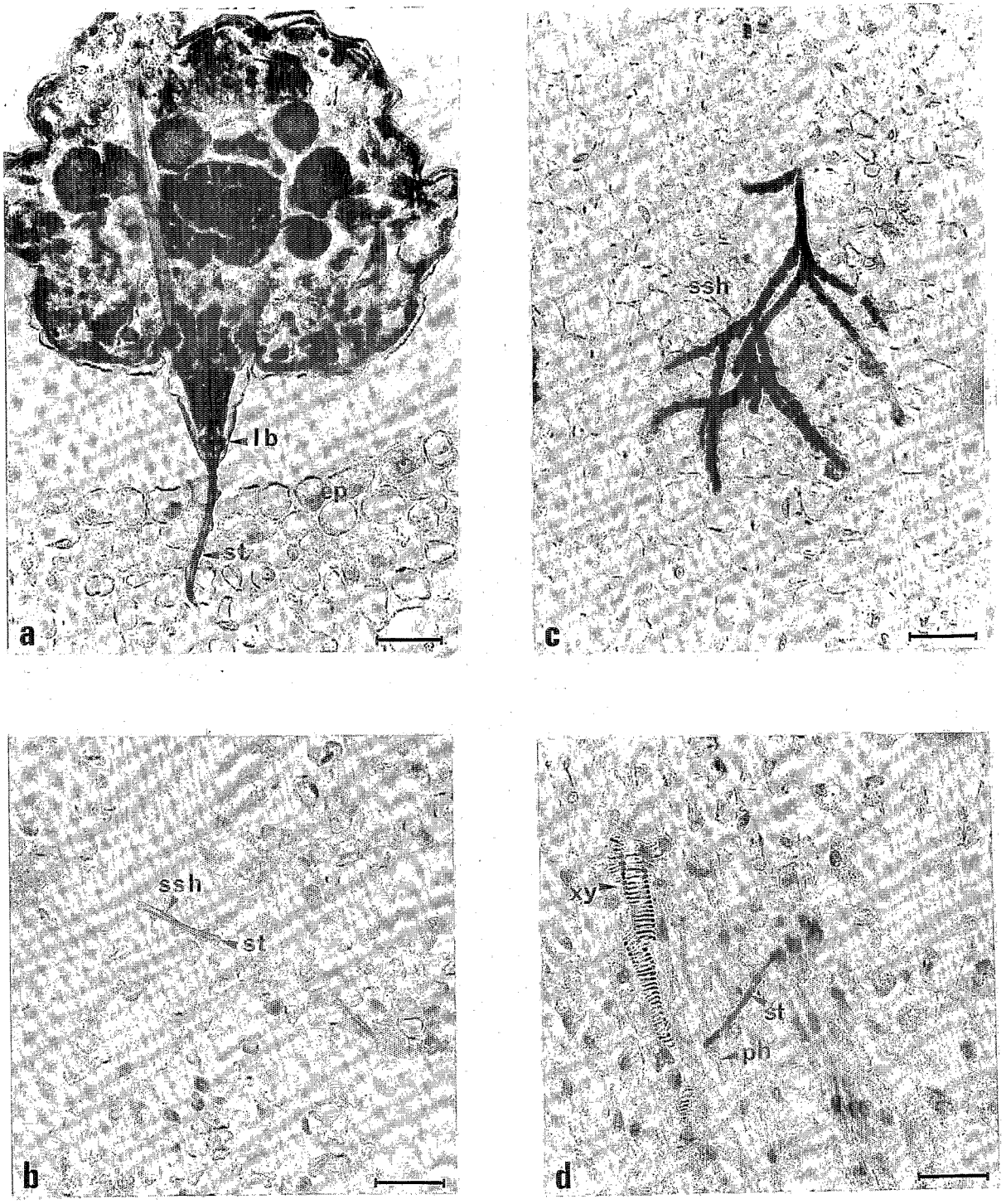

Fig. 1. Light microscopy of stylet pathways of *P. manihoti* feeding on apical stem of *Manihot esculenta* cv. M'pembe. a. Longitudinal section of labium (lb), stylets (st) and transversal section of host epidermis (ep). b. Transverse section of stem showing an extracellular pathway for stylets (st) and sheath (ssh) in parenchyma. c. Transverse section of stem showing a complex branched stylet sheath. d. Longitudinal section of stem showing xylem veins (xy) and a stylet penetration in a phloem cell (ph). Bar = 10 μm.

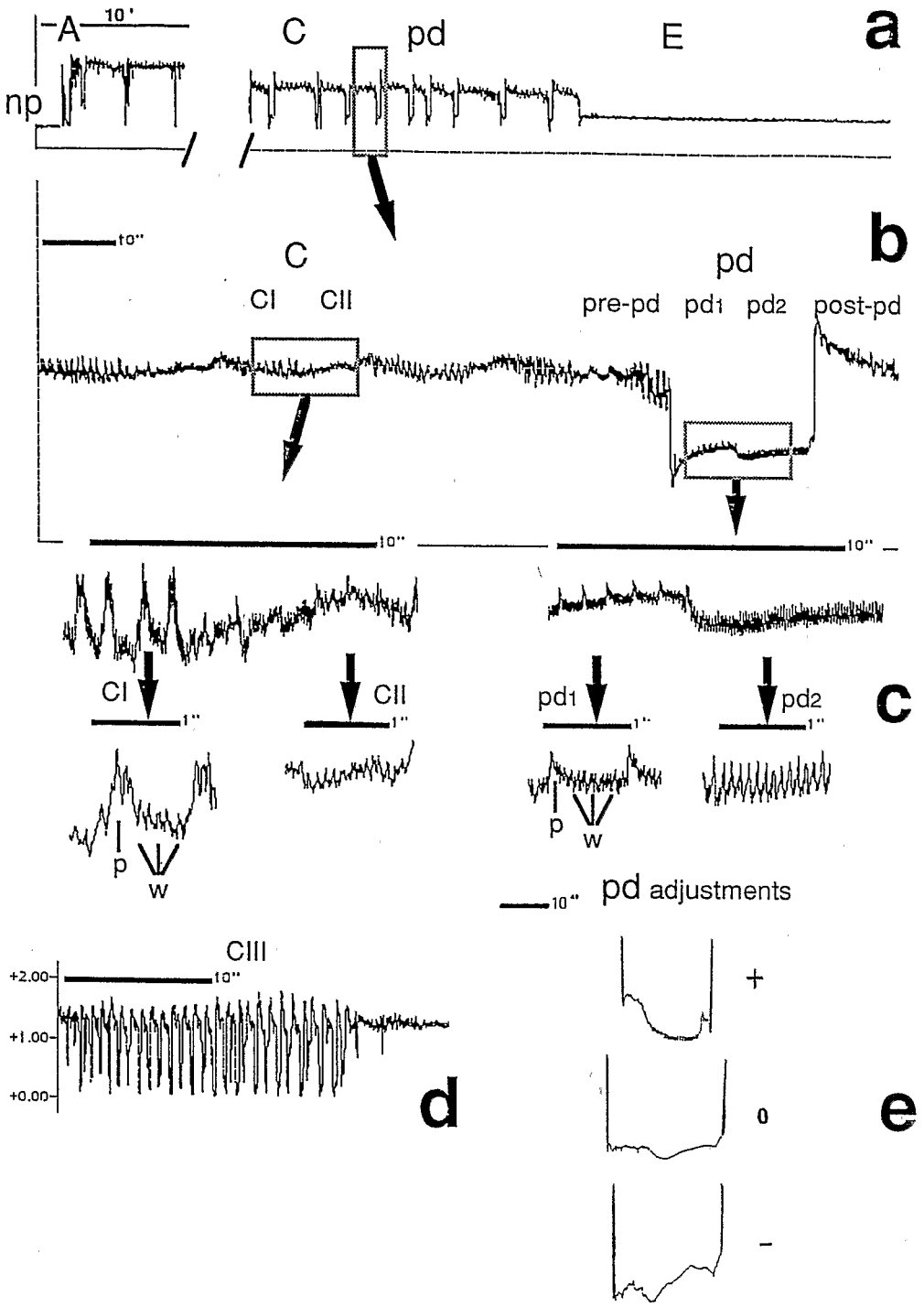

Fig. 2. Cassava mealybug EPG patterns. a. Model one hour recording, showing a sequence of contact (A), cell-wall activities (C), cell punctures (pd) and phloem cell ingestion (E). b. Zooming in around an intracellular puncture, showing subpatterns within C and pd. c. Further zooming showing periodic elements within patterns CI, CII, pd1 & pd2 (p = peaks, w = waves). d. Occasionally occurring CIII pattern; note the zero Volt limit of the downward peaks (see text). e. pd adjustments, displaying changes in pd shape in response to varying input voltage (from + on top to – on bottom).

been done during these transient elements, one may nevertheless interpret the shifts to the 0 V level as notable increases of the insect-plant resistance component of the signal, as if either a resistive plant tissue were briefly reached, or a dramatic constriction of the electrical- i.e. alimentary-duct occurred.

As an intrinsic part of pattern C, the potential drops (pd) occurred. They consisted of sudden drops in the voltage level (Fig. 2a and 2b), maintained for about 20 s (mean, SE = 0.6 s, n = 20), and a fast return to the original level. When the plant voltage was changed from the usual positive adjustment (Tjallingii, 1985a) to about 0 V, then to negative, the sign and magnitude of the pd largely remained unaltered (Fig. 2e), demonstrating that the pd was due to electromotive force (emf) and not to conductance fluctuations in the insect-plant circuit. This proved that it matched the pd features from aphids, presumably caused by the transmembrane potential of a plant cell when punctured by the stylets. During the lowered level of the pd, two characteristic sub-patterns were shown, one of emf origin (pd1, Fig. 2b and 2c) showing peaks at about 1–2 Hz, and another less clear pattern, showing a lower amplitude and a higher frequency (pd2, Fig. 2b and 2c), presumably due to conductance as well as to emf. In contrast to the situation described with pattern CI/CII, the high frequency elements (waves) of patterns pd1 and pd2 are clearly distinct, suggesting that a totally different 'behaviour' is probably performed during these two sub-patterns. For the two other Pseudococcidae used, (*F. virgata* and *R. invadens*), mean pd duration were respectively of 15.1 s (n = 8, SE = 0.5) and 19.8s (n = 6, SE = 1.4). With *P. manihoti*, a maximum of 30 pd's was recorded before reaching the next EPG pattern, waveform E. In aphid EPGs a considerably higher number of pd's was often found (Kimmins & Tjallingii, 1985; Tjallingii, 1985b), with about 1 min as an average interval between pd's (Montllor & Tjallingii, 1989; Hogen Esch & Tjallingii, 1992). Together with the quite longer duration of mealybug pd's, compared to aphids' 5–7 seconds, this point is the main difference between the cell-puncturing behaviours of the two Homopteran families.

Following pattern C, pattern E started with a drop of the voltage level (Fig. 2a and 3), as in the pd, but the lowered level was maintained throughout the whole pattern, indicating sustained intracellular position of the stylet tips while the membrane potential remained intact. Honeydew secretion was always observed during this pattern. These observations strongly suggest that pattern E represents a sieve element puncture with sap ingestion, rather comparable to aphid EPGs. Although not studied precisely here, we distinguished also two sub-patterns E1 and E2. E1 consisted of rather unstable elements lasting variable times but generally less than 2 min. It frequently contained square-shaped peaks (Fig. 3b), leading progressively to the stable periodic phase E2. E1 showed some similarity with the first period in the pd signal (pd1, see above), however, this similarity needs further investigation to verify any suggestion to a similar insect activity. The waveforms during the stable phase E2 often showed two different phases occurring in alternating periods: E2I with low peaks at about 1–3 Hz, due to emf (peaks not inverted with voltage adjustment, see Fig. 3c), and E2II with small irregular waves due to conductance and emf (adjustments not shown). Though E2I was predominant, and sometimes occurring alone, frequent alternations with E2II occurred (Fig. 3). This again seems to be a distinctive feature of mealybugs; aphids possess an E1 phase (with emf peaks) preceding E2 for only about 1 min, and a simpler E2 pattern (phloem sap ingestion, displaying a large conductance component) and no E2II equivalent. We must mention that the E1/E2 homology between aphids and mealybugs, reflected by a common terminology, remains loose until more is known of mealybug ingestion phases.

Other aphid patterns (F and G), were not clearly observed in our EPGs on mealybugs. However, extensive recordings are still required to detect or rule out their occurrence in coccids, as short G-resembling phases were sometimes observed. Similarly, phases resembling E2II patterns were occasionally detected at extracellular voltage levels, and could be related to the still unclear E(c) pattern of aphids.

Comparative EPGs on different hosts. Table 3 presents the different EPG parameters obtained on the six hosts studied. Time to phloem access (t-E) was not included as it was essentially similar to t-Ei, implying that ingestion almost always occurred i.e. access was always longer than 10 min) when phloem was discovered. Kruskal-Wallis tests on pd duration showed significant differences between plants (and also for phases 1 and 2 inside pd's). Whether this is related to host and non-host status remains unclear: Cassavas induce the longest pd duration (20–21 s) while substitute plants induce the shortest (17–18 s), and the 'Faux-caoutchouc' hybrid intermediate values. Although the differences are small, they are significant according to a Kruskal & Wallis test (Table 3). For numbers of non-

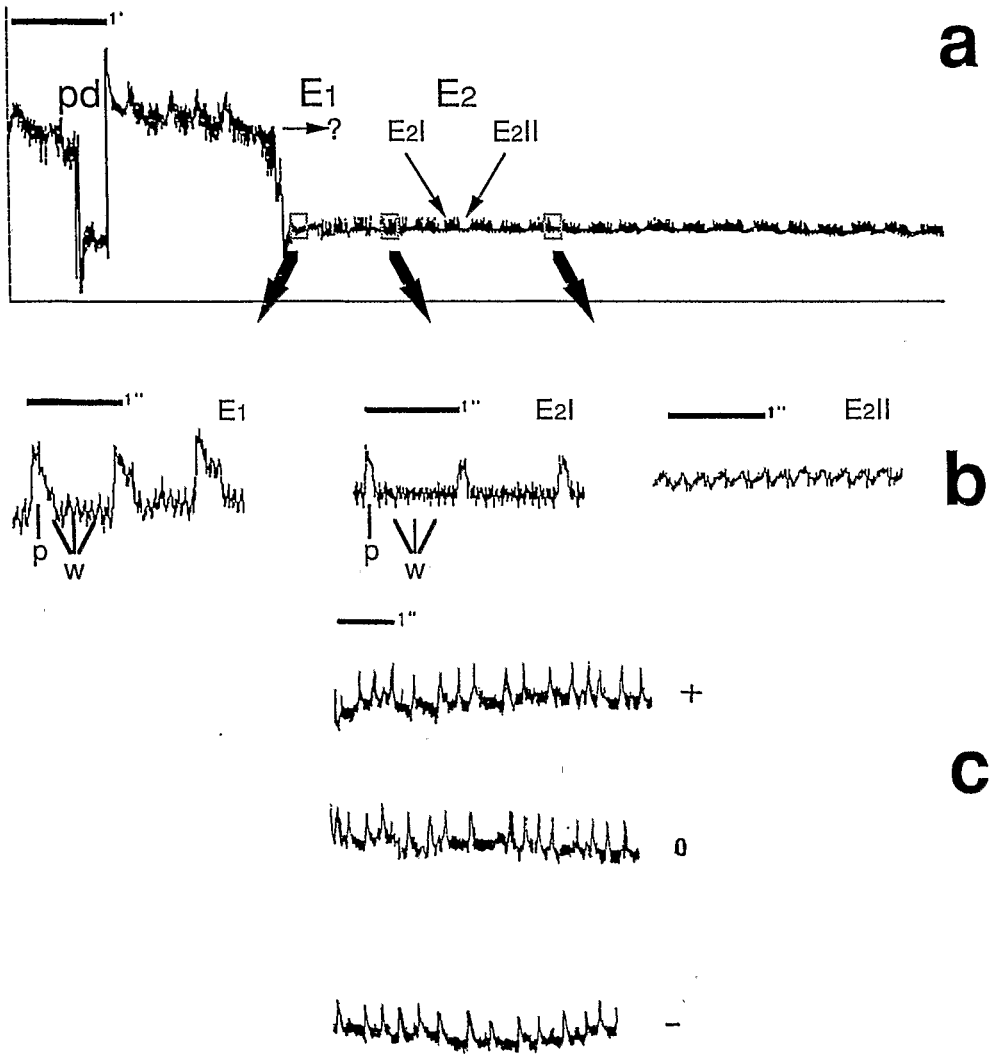

Fig. 3. Details of pattern E from cassava mealybug. a. Last pd and sustained puncture of a phloem element, leading to subsequent ingestion. b. Zooming in of the top squared portions displaying details and constitutive periodic elements of pattern E (p = peaks, w = waves). c. Voltage adjustments within E2I pattern, resolving the emf nature of the peak elements.

penetration intervals, *i.e.* total number of probes, hosts scored lower than non-host plants. The first 10 h of EPGs showed even clearer differences between hosts and non-hosts (not shown). On the whole, host plants showed less pathway activity (pattern C), fewer probes, and especially fewer probes without a sieve element puncture. In spite of these differences, no significant correlation could be found between susceptibility index numbers and any of these EPG parameters when all plants were included. Nevertheless, parameters such as time to first E, time to sustained E, and duration of E, showed a clear (rank) correlation within the 3 cassava cultivars with the susceptibility index. The other 3 plants apparently did not fit into this order, thus exemplifying that a synthetic index such as the susceptibility index could not be considered as linearly determined by any of the simple behavioural figures analysed.

Leaf chemicals. Alkaloids, phenolic compounds (phenolic acids and flavonoids) and free cyanides were assayed in putative phloem sap and crude extracellular fluids (see Materials and methods).

Table 3. Means of EPG parameters of cassava mealybug on the six host plants studied. Data presented as mean ± SE (n). nd= not determined

Plant susceptibility index* n=(except pd) EPG parameters**	Poinsettia 0 (4)	Talinum 0 (6)	Incoza 1 (6)	Zanaga 2 (3)	30M7 3 (4)	Faux caoutchouc 4 (6)	p of test*** (Kruskal-Wallis)
on whole recordings (var. times)							
t-Ei	9.2 ± 3.1	10.8 ± 3.5	7.9 ± 4.3	4.9 ± 1.2	1.9 ± 0.4	3.9 ± 0.8	0.46
n-E	3.0 ± 1.1	1.2 ± 0.6	5.5 ± 2.2	4.0 ± 1.7	4.0 ± 2.8	2.1 ± 0.7	0.45
d-E	0.2 ± 0.1	1.6 ± 1.0	3.8 ± 1.2	4.0 ± 0.3	5.1 ± 3.1	2.6 ± 1.5	0.47
ds-pd	ab 18.5 ± 0.6 (25)	a 17.2 ± 1.1 (15)	nd	bc 20.4 ± 0.6 (25)	c 21.2 ± 1.1 (12)	bc 19.1 ± 0.5 (25)	0.0087
ds-pd1	b 10.0 ± 0.3 (19)	a 8.6 ± 0.4 (15)	nd	ab 9.4 ± 0.3 (16)	c 11.5 ± 0.6 (12)	b 9.8 ± 0.3 (24)	0.0001
ds-pd2	a 8.1 ± 0.5 (19)	a 8.0 ± 0.9 (15)	nd	b11.0 ± 0.7 (16)	ab 9.7 ± 1.3 (12)	a 8.2 ± 0.4 (24)	0.029
d-C	9.9 ± 2.7	11.4 ± 3.4	9.7 ± 2.4	4.5 ± 0.4	6.0 ± 2.9	6.7 ± 1.6	0.52
n-np	11.7 ± 4.9	9.3 ± 2.9	5.1 ± 1.7	0	2.2 ± 1.0	5.3 ± 1.6	0.065
d-np	1.3 ± 0.4	1.4 ± 0.6	1.7 ± 0.8	0	0.5 ± 0.2	0.4 ± 0.2	0.089
n-pen(E)	1.5 ± 0.3	0.8 ± 0.4	1.8 ± 0.7	1	0.7 ± 0.5	1.0 ± 0.2	0.68
n-pen(xE)	d 10.2 ± 4.7	c 9.2 ± 2.2	bcd 3.5 ± 1.3	a 0	ab 1.2 ± 0.6	abd 4.6 ± 1.3	0.026
d-pen(E)	3.6 ± 0.6	3.3 ± 2.0	11.4 ± 2.8	17.5 ± 4.3	11.7 ± 6.9	7.0 ± 2.2	0.15
d-pen(xE)	b 7.8 ± 3.6	b 10.9 ± 2.5	ab 6.3 ± 2.4	a 0	a 0.8 ± 0.6	ab 3.0 ± 0.7	0.035
on first 10 hours							
d-C-10	c 5.86 ± 1.21	c 5.57 ± 0.77	bc 3.69 ± 0.49	ab 2.53 ± 0.91	a1.56 ± 0.40	bc 4.28 ± 0.41	0.020
n-np-10	c 9.00 ± 3.71	bc 6.00 ± 1.80	ab 2.50 ± 0.76	a 0	a 0.25 ± 0.25	abc 3.83 ± 1.40	0.017
d-np-10	1.01 ± 0.32	0.96 ± 0.38	0.72 ± 0.35	0	0.24 ± 0.24	0.34 ± 0.19	0.073
n-pen(E)-10	1.00 ± 0.40	0	1.00 ± 0.51	1	0.50 ± 0.28	1.00 ± 0.25	0.086
n-pen(xE)-10	c 8.25 ± 3.74	bc 6.86 ± 1.49	a 2.16 ± 0.83	a 0	a 0.25 ± 0.25	ab 3.50 ± 1.23	0.011
d-pen(E)-10	ab 1.39 ± 0.78	a 0	ab 3.67 ± 1.83	c 9.71 ± 0.29	bc 5.00 ± 2.88	bc 4.79 ± 1.07	0.031
d-pen(xE)-10	bc 5.55 ± 2.16	c 6.55 ± 1.36	abc 3.94 ± 1.59	a 0	a 0.04 ± 0.04	ab 2.37 ± 0.75	0.022

* see Materials and methods

** see Table 1

*** For each line, when $p<0.05$, the range values of a Fisher-PLSD test are given. Means preceded by the same letter are not different at the 5% level.

Alkaloids were not detected in any fluid from all the plants analysed. Phenolic acids and flavonoids were detected in phloem sap and extracellular fluids (among which the glycosylated flavonoid rutin was identified in both extracts). Phenolic profiles differed readily between these two cellular compartments (data not shown and not discussed here). Free cyanides were absent from phloem sap but present in extracellular fluids. In contrast, bound cyanides were found in both fluids and thus found to be effectively translocated in phloem sap (data not shown).

In extracellular fluids, flavonoid levels showed no significant differences between plants (mean values around 10 mg/g dry weight of extract), but cyanides and phenolic acids discriminated hosts from non-hosts, as well as between different hosts. No cyanides were found in non-host plants (poinsettia and *Talinum*), and cyanide values differed significantly between *Manihot* varieties [Incoza ≤ Faux Caoutchouc ≤ Zanaga < 30M7; values of free cyanides in mg/g dry weight of extract respectively of: 0.5 ± 0.1(a), 0.62 ± 0.1(ab), 0.87 ± 0.3(b), 2.1 ± 0.5(c); Anova $p<0.0001$ and letters from Fisher's PLSD test]. On the contrary, phenolic acids were found to be higher in non-hosts than in hosts [30M7 ≤ Incoza ≤ Zanaga ≤ Faux Caoutchouc ≤ Talinum ≤ Poinsettia; respective values, in mg/g, of: 0.69 ± 0.1(a), 1.18 ± 0.1(ab), 1.24 ± 0.1(ab), 1.45 ± 0.1(b), 2.05 ± 0.3 (bc), 2.55 ± 0.3(c); Anova $p<0.0001$, letters from Fisher's PLSD test].

Conclusions

Light microscopy and EPG recordings of *P. manihoti* show similarities with stylet penetration of other phloem-feeding Homoptera, such as aphids or whiteflies (Pollard, 1973; Tjallingii, 1978; Janssen *et al.*, 1989). How these Homoptera locate the sieve tubes is still not clearly understood, but the guiding factors are probably a combination of physical and chemi-

cal stimuli (Rahbé *et al.*, 1993). The Pseudococcidae *P. manihoti* displays a typical extracellular penetration behaviour, with secretion of a branched salivary sheath inducing little cell damage, and probably leading in most cases to a phloem vessel. The role of saliva in Homoptera feeding is very important, since it is thought to act as a carrier of enzymes and gustatory stimuli, and to help stylet penetration in forming a multi-functionnal stylet sheath (Miles, 1972). Light microscopy alone cannot assess the actual stylet pathways, since putative intracellular paths were shown to be in fact intramural/extracellular by accurate TEM examination (Spiller *et al.*, 1985). However, as far as we can rely on EPG traces, *P. manihoti* seems to display an exclusively extracellular route to phloem, with periodic intracellular punctures identified by the potential drops. Intracellular pathway should theoretically be reflected in EPGs by abnormal i.e. non-pd, non-E) potential shifts, but this remains to be tested experimentally.

The comparison of EPGs from mealybugs (mainly *P. manihoti*) with those from aphids shows some differences that might be significant in distinguishing their interactions with host plants. The main differences concern i) duration of pds (≈20 s in mealybugs including *F. virgata* and *R. invadens*, ≈5–7 s in aphids), ii) the number of pds/time unit of pattern C (recurrence of pds much lower in mealybugs, 15–20/h on all plants, than in aphids, 50–60/h), iii) *minimal* time to reach the phloem — not mean time — (1.5 h on favourable host and 2.8 h on substitute host for *P. manihoti*, often less than 15 min for aphids), iv) the number and success of penetrations, i.e. the fact that the phloem is reached during a single penetration (van Helden & Tjallingii, 1993), emphasising the lower mobility of mealybugs within their feeding sequence, v) the complex structure of the supposed 'sieve-element ingestion' pattern with occasional alternation of two distinct phases of unknown significance (E2I and E2II). From this initial study, it is difficult to speculate on the consequences of these features, but the much longer intracellular contacts within pds, the lower pd recurrence, intermediate between that of aphids and whiteflies (Janssen *et al.*, 1989), as well as the long time period spent in extracellular spaces might be important to have in mind when comparing mealybugs with aphids, either in terms of behavioural analysis or in terms of chemical ecology. It is worth mentioning that coccids, as it is the case for aphids or other Homoptera, do not possess chemosensors on their stylet tips but rely on the specialised precibarial receptors for gustatory discrimination (Pesson, 1944; Backus, 1988). This does not preclude the presence of labial tip chemoreceptors that may be important for host surface selection, as it occurs in some other Homoptera (Walker & Gordh, 1989).

Comparing behaviour of *P. manihoti* on the different host plants tested leads to significant correlations between some parameters and host status (host *vs* non-host, or related to susceptibility index). Over the whole series of plants, host status is linked with phloem access, although this parameter is quite variable within hosts (Spearman's rank test between index of susceptibility and t-Ei, $\rho = -0.928$, $p = 0.038$). This suggests that in the range tested, pre-phloem interactions are most important for host-plant acceptance, and that early plant rejection due to delay in phloem finding may result in global 'antixenosis'. On non-host plants, low acceptance is linked to considerable increase in penetration 'failures' (greater n-pen(xE), see Table 3). Finally, durations of potential drops, and thus of intracellular punctures, are substantially shorter on non-host plants, especially on *Talinum*. This could be due to physical differences in cell or tissue structures, but may also reflect a differential response to intracellular chemical cues (pH, nutrients, secondary plant compounds), either positive in cassava hosts or slightly negative in substitute plants. The physiological implications of such observations will not be fully exploited until more is known about the functional correlations within pds (correlations of sub-patterns with precise behavioural sequences such as salivation and ingestion).

Concerning the chemistry of leaf fluids, it was clear that total flavonoids were not correlated with any of the individual probing parameters nor with susceptibility index (all rank correlations <0.5), indicating that these components as a whole were not involved in the initial interaction between the mealybug and its host. Such a situation was already observed in a hypersensitive reaction of peach tree to the aphid *Myzus persicae*, where flavonoids were not affected by the clear oxydative burst following aphid attack (Rahbé *et al.*, 1988), but cannot be generalised, even to other phloem-feeders (Klingauf, 1971; Kim *et al.*, 1985; Mullin, 1986).

On the contrary, phenolic acid levels are strongly correlated with parameters such as d-E ($r = -0.988$; Spearman's test, $p = 0.035$), d-C-10 ($r = +0.938$; $p = 0.035$) or n-np-10 ($r = 0.947$; $p = 0.063$). On the cultivar 30M7, which has the lowest phenolic acid level in its extracellular fluids, aphids spent the

least time searching for the phloem (t-Ei <2 h, d-C-10 = 2.5 h). Although correlation is not proof of causality, this fact is interesting in view of the role of phenolic acids in cell-wall structure, as precursors of lignins, cutins/suberins or phenolic-coupled pectins that could interact with salivary oxidising enzymes (Fry, 1983; Goodman, 1986).

Cyanide compounds are present in the vacuolar compartment of meristem tissues of cassava in the bound form of the glucosides linamarin and lotaustralin (Conn, 1980). In *Phaseolus lunatus*, it has been shown that free cyanides were liberated by an apoplastic enzyme called linamarase (Frehner & Conn, 1987). This localisation was confirmed in cassava (Mkpong *et al.*, 1990), explaining the occurrence of free cyanides in extracellular fluids of cassavas and not in phloem sap. When correlating cyanide contents with EPG parameters, the best rank correlation clearly identified ds-pd, the duration of intracellular punctures (ρ = 0.975, p = 0.05). This is a possible indication of the use of cyanides or cyanogenic compounds as allelochemicals for host recognition during cell penetration by *P. manihoti*. However, as for phenolic acids, the present observations are not sufficient to conclude this with certainty, and the correlation between the two classes of substances in our series of cultivars (r = −0.871) needs to be avoided in further experiments if we want to distinguish the respective effects of these metabolites on the probing behaviour of cassava mealybug. In addition, the low number of replicates in our EPG trials affects the quantitative significance of the resulting parameters, and may lead to inconsistencies in some correlations. Increasing the number of replicates seems essential in further tests.

In conclusion, the use of electrical recordings in applied research such as plant resistance analysis implies an unambiguous correlation between the recorded patterns and elements of the stylet-penetration behaviour (Tjallingii, 1990), especially when dealing with new insect groups. We initiated this for the cassava mealybug in the present study. The significance of new EPG waveforms (i.e. non-aphid patterns) may also be clarified by a better understanding of the electrical origins of the signal, which combines the activities of the penetrating animal (e.g. saliva secretion, fluid intake or penetration efforts) and the location of the stylet tips in plant cells and tissues (Tjallingii, 1987; Janssen *et al.*, 1989). Comparative data on the details of feeding behaviour in different families of Homoptera are not only important in HPR research, but also for example in studies on the transmission of viruses or other plant pathogens by these insects.

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References

Albrigo, L. G. & R. F. Brooks, 1977. Penetration of citrus cuticles and cells by citrus snow scale, *Unaspis citri* (Comst.). Proc. Int. Soc. Citriculture 2: 463–467.

Auclair, J. L., 1988. Host plant resistance. In: A. K. Minks & P. Harrewijn (Eds), Aphids, their biology, natural enemies and control. Elsevier, Amsterdam: pp. 225–265.

Backus, E. A., 1988. Sensory systems and behaviours which mediate hemipteran plant-feeding: a taxonomic overview. J. Insect Physiol. 34: 151–165.

Bounias, M., 1983. Analyse biochimique quantitative par nanochromatographie en couche mince. Masson, Paris (FRA): 198 pp.

Boussienguet, J., 1984. Bioecologie de la cochenille du manioc *Phenacoccus manihoti* Matile-Ferrero et de ses ennemis naturels au Gabon. Univ. Thesis, Fac. Sci. Paris IV: 100 pp.

Campbell, C. A. M., 1990. The susceptibility of cocoa to mealybugs (Pseudococcidae) and other honeydew-producing Homoptera in Ghana. Bull. entomol. Res. 80: 137–151.

Conn, E. E., 1980. Cyanogenic compounds. Annu. Rev. Plant Physiol. 31: 433–451.

Dreyer, D. L. & K. C. Jones, 1981. Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae*: aphid feeding deterrents in wheat. Phytochemistry 20: 2489–2493.

Dreyer, D. L., K. C. Jones & R. J. Molyneux, 1985. Feeding deterrency of some pyrrolizidine, indolizidine and quinolizidine alkaloids towards pea aphids (*Acyrthosiphon pisum*) and evidence for phloem transport of indolizidine alkaloid swainsonine. J. chem. Ecol. 11:1045–1050.

Dreyer, D. L. & B. C. Campbell, 1987. Chemical basis of host-plant resistance to aphids. Plant Cell Environ. 10: 353–361.

Emden, W. F. van & C. H. Wearing, 1965. The role of host plant in delaying economic damage levels in crop plants. Ann. appl. Biol. 56: 323–324.

Fraenkel, G., 1969. Evaluation of our thoughts on secondary plant substances. Entomol. exp. appl. 12: 473–486.

Frehner, M. & E. E. Conn, 1987. The linamarin β -glucosidase in Costa Rican Wild Lima beans (*Phaseolus lunatus* L.) is apoplastic. Plant Physiol. 84: 1296–1300.

Fry, S. C., 1983. Feruloylated pectines from the primary cell wall: their structures and possible functions. Planta. 157: 111–123.

Gabe, M., 1968. Techniques histologiques. Masson, Paris (FRA): 1113 pp.

Goodman, R. N., 1986. Cell-wall composition and metabolism. In: R. N. Goodman (Eds), The Biochemistry and Physiology of Plant disease. University of Missouri Press, Columbia (USA): 105–149.

Helden, M. van & W. F Tjallingii, 1993. The resistance of lettuce (*Lactuca sativa* L.) to *Nasonovia ribisnigri*: the use of electrical penetration graphs to locate the resistance factor(s) in the plant. Entomol. exp. appl. 68: 269–278.

Herren, H. R., 1981. Biological control of the cassava mealybug. Tropical Root Crops, Research Strategies for the 1980s – First Triennal Root Crops Symposium, Ibadan (NIG), International Institute of Tropical Agriculture: 79–80.

Herren, H. R., 1987. Africa-wide biological control project of cassava mealybug and cassava green mites: a review of objectives and achievements. Ins. Sci. Appl. Sp. issue 'Africa-wide Biological Control Project of Cassava Pests': 837–840.

Hogen Esch, T. & W. F. Tjallingii, 1992. Ultrastructure and electrical recording of sieve elements punctures. In: S. B. J. Menken, J. H. Visser & P. Harrewijn (Eds), 8th Int. Symp. Insect-Plant Relationships, Wageningen (NDL), Kluwer Acad. Publ.: pp. 283–285.

Janssen, J. A. M., W. F. Tjallingii & J. C. van Lenteren, 1989. Electrical recording and ultrastructure of stylet penetration by the greenhouse whitefly. Entomol. exp. appl. 52: 69–81.

Kim, M., H. S. Koh & H. Fukami, 1985. Isolation of C-Glycosylflavones as probing stimulant of planthoppers in rice plant. J. chem. Ecol. 11: 441–452.

Kimmins, F. M. & W. F. Tjallingii, 1985. Ultrastructure of sieve element penetration by aphid stylets during electrical recording. Entomol. exp. appl. 39: 135–141.

Klingauf, F. A., 1971. Die Wirkung des Glucosids Phloridzin auf das Wahlverhalten von *Rhopalosiphum insertum* (Wald.) und *Aphis pomi* de Geer (Homoptera: Aphididae). Z. angew. Entomol. 68: 41–55.

Kogan, M., 1977. The role of chemical factors in insect/plant relationships. Proc. XV Int. Congr. Entomol., Washington (USA), Academic press, New York: pp. 211–227.

Kogan, M. & E. F. Ortman, 1978. Antixenosis, a new term proposed to replace Painter's 'non-preference' modality of resistance. S.A.E. Bull. 24: 175–176.

Leszczynski, B., J. Warchoe & S. Niraz, 1985. The influence of phenolic compounds on the preference of winter wheat cultivars by cereal aphids. Insect Sci. Appl. 6: 157- –158.

Locquin, M. & M. Langeron, 1978. Manuel de microscopie. Masson, Paris (FRA): 352 pp.

Matile-Ferrero, D., 1976. Les cochenilles nuisibles au manioc en république populaire du Congo. Muséum National d'Histoire Naturelle, mission report Oct 1975: 30 pp.

Mc Foy, C. C. A. & Z. T. Dabrowski, 1984. Preliminary studies on cowpea resistance to *Aphis craccivora* Koch (Hom., Aphididae). Z. ang. Entomol. 97: 202–209.

Miles, P. W., 1972. The saliva of Hemiptera. Adv. Insect Physiol. 9: 183–255.

Mkpong, O. E., H. Yan, G. Chism & R. Sayre, 1990. Purification, characterisation and localisation of linamarase in cassava. Plant Physiol. 93: 176–181.

Molyneux, R. J., B. C. Campbell & D. L. Dreyer, 1990. Honeydew analysis for detecting phloem transport of plant natural products. Implications for host-plant resistance to sap-sucking insects. J. chem. Ecol. 16: 1899–1910.

Montllor, C. B. & W. F. Tjallingii, 1989. Stylet penetration by two aphid species on susceptible and resistant lettuce. Entomol. exp. appl. 52: 103–111.

Mullin, C. A., 1986. Adaptive divergence of chewing and sucking arthropods to plant allelochemicals. In: L. B. Brattsten & S. Ahmad (Eds), Molecular aspects of insect-plant associations. Plenum, pp. 175–209.

Neuenschwander, P., F. Schulthess & E. Madojemu, 1986. Experimental evaluation of the efficiency of *Epidinocarsis lopezi*, a parasitoid introduced into Africa against the cassava mealybug *Phenacoccus manihoti*. Entomol. exp. appl. 42: 133–138.

Painter, R. H., 1958. Insect resistance in crop plants. Annu. Rev. Entomol. 3: 267–290.

Pereira, J. F. & W. E. Splittstoesser, 1987. Exudate from cassava leaves. Agric. Ecosystems Environ. 18: 191–194.

Pesson, P., 1944. Contribution a l'étude morphologique et fonctionnelle de la tête, de l'appareil buccal et du tube digestif des femelles de Coccides. Monographie CNRA/INRA, Versailles (FRA): 266 pp.

Pickett, J. A., L. J. Wadhams, C. M. Woodcock & J. Hardie, 1992. The chemical ecology of aphids. Annu. Rev. Entomol. 37: 67–90.

Pollard, D. G., 1973. Plant penetration by feeding aphids (Hemiptera: Aphidoidae): A review. Bull. entomol. Res. 62: 631–714.

Rahbé, Y., B. Delobel, G. Febvay & G. Massonie, 1988. Résistance de certaines variétés de Pêchers *Prunus persica* au puceron *Myzus persicae* (Homoptera): rôle des composés phénoliques. Ann. Soc. entomol. Fr. 24: 472.

Rahbé, Y., G. Febvay, B. Delobel & G. Bonnot, 1993. Amino acids and proteins as cues in aphid-plant interactions. Symp. on Homopteran feeding behaviour. Proc. XX Int. Congr. Entomology, Beijing (CHI), 28/06–04/07 1992 G. Walker (ed.) (in press).

Rohringer, R., F. Ebrahim-Nesbat & G. Wolf, 1983. Protein in intercellular washing fluids from leaves of Barley (*Hordeum vulgare*). J. exp. Bot. 34: 1589–1605.

Schoonhoven, L. M. & I. Derksen-Koppers, 1976. Effects of some allelochemicals on food uptake and survival of polyphagous aphid, *Myzus persicae*. Entomol. exp. appl. 19: 52–56.

Smith, B. D., 1966. Effect of plant alkaloid sparteine on the distribution of the aphid *Acyrthosiphon spartii* (Koch). Nature. 212: 213–214.

Spiller, N. J., F. M. Kimmins & M. Llewellyn, 1985. Fine structure of aphid stylet pathways and its use in host plant resistance studies. Entomol. exp. appl. 38: 293–295.

Starks, K. J., R. Muniappan & R. D. Eikenbary, 1972. Interaction between plant resistance and parasitism against the greenbug on barley and sorghum. Ann. entomol. Soc. Am. 65: 650–655.

Tertuliano, M., S. Dossou-Gbete & B. Le Rü, 1993. Antixenotic and antibiotic components of resistance to the cassava mealybug *Phenacoccus manihoti* (Hom. Pseudococcidae) in various host plants. Insect Sci. Appl. (14(4)(in press).

Tjallingii, W. F., 1978. Electronic recording of penetration behaviour by aphids. Entomol. exp. appl. 24: 521–530.

Tjallingii, W. F., 1985a. Electrical nature of recorded signals during stylet penetration by aphids. Entomol. exp. appl. 38: 177–186.

Tjallingii, W. F., 1985b. Membrane potentials as an indication for plant cell penetration by aphid stylets. Entomol. exp. appl. 38: 187–193.

Tjallingii, W. F., 1987. Stylet penetration activities by aphids: new correlations with electrical penetration graphs. In: V. Labeyrie, G. Fabres & D. Lachaise (Eds), Insects-Plants. Dr W. Junk Publishers, pp. 301–306.

Tjallingii, W. F., 1988. Electrical recording of stylet penetration activities. In: A. K. Minks & P. Harrewijn (Eds), Aphids, their

Biology, Natural enemies and Control. Elsevier, Amsterdam (NDL): 95–108.

Tjallingii, W. F., 1990. Stylet penetration parameters from aphids in relation to host-plant resistance. Symp. Biol. Hung. 39: 411–419.

Todd, G. W., A. Getahun & D. C. Cress, 1971. Resistance in barley to the greenbug, *Schizaphis graminum*. 1. Toxicity of phenolic and flavonoid compounds and related substances. Ann. entomol. Soc. Am. 64: 718–722.

Walker, G. P. & G. Gordh, 1989. The occurrence of apical labial sensilla in the Aleyrodidae and evidence for a contact chemosensory function. Entomol. exp. appl. 51: 215–224.

Wink, M. & L. Witte, 1991. Storage of quinolizidine alkaloids in *Macrosiphum albifrons* and *Aphis genista* (Homoptera, Aphididae). Entomol. general. 15: 237–254.