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Cassava-Mealybug Interactions

Paul-André Calatayud

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Cassava–Mealybug Interactions

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Introduction

Mealybugs are scales of the Pseudococcidae family and members of the Sternorrhyncha (formerly Homoptera) order. They are so called due to the thin-to-thick mealy or cottony wax secretion covering the insects. Mealybugs are serious pests of fruit trees, ornamentals, food crops and many other cultivated plants. Their morphology, classification, biology, habitats, genetic systems and control measures, including the more recent use of pheromone traps, are well described in the literature. Although a great number of studies have been carried out on basic plant–aphid interactions of Sternorrhyncha (formerly Homoptera), plant–mealybug interactions are poorly described. Most basic information on plant–mealybug interactions during the last decade has come from research on the cassava, *Manihot esculenta* Crantz (Euphorbiaceae) system with two mealybug species, namely *Phenacoccus manihoti* Matile-Ferrero and *Phenacoccus herreni* Cox and Williams (Sternorrhyncha: Pseudococcidae). Both these Pseudococcidae species cause severe damage to cassava in Africa and South America, respectively.

This book reviews these mealybug–plant interactions. It briefly introduces the cassava plant and describes some aspects of the biology, systematics and distribution of the two mealybug species. The host plant selection behaviour of these mealybugs is then reviewed, presenting their sensory systems, selection and feeding behaviours. Their nutritional physiology is mentioned through the description of their saliva, the biochemical constituents of the ingesta, and the digestive enzymes, thus providing an insight into mealybug nutritional requirements. The influence of climate on cassava–mealybug interactions is also discussed. In addition, plant-defence mechanisms that play a role in host-plant resistance are presented. Intrinsic mechanisms including antixenosis, antibiosis and tolerance, and extrinsic mechanisms relating to active interactions between mealybug-infested plants and the third trophic level of insect parasitoids and predators are discussed.

Published as well as unpublished works are reviewed. In the case of the latter, results are given in more detail and the authors' contributions, as well as the materials and methods used, are presented. In general, this review offers an insightful and analyti-

cal perspective of cassava–mealybug interactions, giving personal views and presentations. We believe our assessments will contribute to a better understanding of these interactions. This book should be useful for entomologists, crop scientists, agronomists and ecologists, among others. The information presented will hopefully stimulate and form a basis for future investigations into this area of research.

Paul-André Calatayud and Bruno Le Rü
Nairobi, July 2006

1 Cassava and mealybugs

THE CASSAVA PLANT

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae) (Figure 1.1), is a perennial root crop native to tropical America and introduced into Africa by the Portuguese in the 1600s. Since that time, cassava has constituted a major food crop for more than 500 million people in the tropical countries of Africa, Asia and Latin America (Cock, 1982). It is cultivated mainly for its starchy storage roots, but also for its leaves, which are high in protein. In Africa, this crop is mostly cultivated as a staple by peasant farmers, whereas in Asia and South America it is also grown on a large scale for starch, fodder and fuel.

Cassava cultivation occurs predominantly in marginal, low-fertility, acidic soils with an annual rainfall ranging from less than 600 mm in the semiarid tropics to more than 1,500 mm in the subhumid and humid tropics (Howeler, 1991) (Figure 1.2). The majority of these regions have an irregular rainfall distribution pattern, generally divided into prolonged dry and wet seasons (El-Sharkawy *et al.,* 1992). In comparison to other staple food crops such as cereals, cassava has a reasonably high productivity under drought and poor soil conditions. Tolerance to prolonged drought is achieved by reduction in the leaf canopy and maintenance of a low but sufficient photosynthetic rate, thereby limiting water loss. Moreover, the plant is capable of slowly extracting deep soil water when it is available (El-Sharkawy, 1993). If the demand for carbohydrates exceeds the supply, however, the plant starts to remobilize carbohydrates from the stems and storage roots to cover respiratory costs and for growth of lateral shoots (Schulthess, 1991; Schulthess *et al.*, 1991).

THE CASSAVA MEALYBUGS

Distribution

Several Pseudococcidae species have been found on *Manihot esculenta* and on other *Manihot* species (Matile-Ferrero, 1977; Cox & Williams, 1981). Williams and

B. Le Rü

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Figure 1.1.
The cassava plant (a) is mostly cultivated for its starchy roots (b)

Figure 1.2.
GIS plot of potential cassava growing areas (defined as having a mean annual rainfall of 500–4,000 mm and mean annual temperature of 19–28°C), and cassava mealybug (*Phenacoccus herreni* and *Phenacoccus manihoti*) distribution according to Williams & Granara de Willink (1992) and Neuenschwander (2003)

Granara de Willink (1992) reported the following 19 species of Sternorrhyncha: Pseudococcidae:

-*Ferrisia meridionalis* Williams
-*Ferrisia terani* Williams & Granara de Willink
-*Ferrisia virgata* (Cockerell)
-*Hypogeococcus spinosus* Ferris
-*Nipaecoccus nipae* (Maskell)
-*Paracoccus herreni* Williams & Granara de Willink
-*Paracoccus marginatus* Williams & Granara de Willink
-*Phenacoccus gregosus* Williams & Granara de Willink
-*Phenacoccus helianthi* (Cockerell)
-*Phenacoccus herreni* Cox & Williams
-*Phenacoccus madeirensis* Green
-*Phenacoccus manihoti* Matile-Ferrero
-*Planococcus citri* (Risso)
-*Planococcus minor* (Maskell)
-*Pseudococcus affinis* (Maskell)
-*Pseudococcus elisae* Borchsenius
-*Pseudococcus mandio* Williams
-*Pseudococcus maritimus* (Ehrhorn)
-*Puto barberi* (Cockerell).

Special attention has been given to *Phenacoccus manihoti* and *Phenacoccus herreni* because these species can cause severe damage to cassava in Africa and South America, respectively (Matile-Ferrero, 1977; Cox & Williams, 1981) (Figure 1.3 a et b). No mealybug has been reported as causing damage on cassava in Asia.

A.C. Bellotti

Figure 1.3 a
Typical curling of cassava canopy leaves caused by mealybug infestation

A.C. Bellotti

Figure 1.3 b
Plant damage symptoms
caused by mealybug infestation

In the early 1970s, *P. manihoti* was accidentally introduced into Africa from its aboriginal home in South America. In the absence of its natural enemies, the mealybug spread rapidly across tropical Africa (Herren, 1981; Herren & Neuenschwander, 1991). In South America, *P. manihoti* is present only in restricted areas of Paraguay, Brazil and Bolivia, while in contrast *P. herreni* is widely distributed in Bolivia, Brazil, Colombia, French Guiana, Grenada, Guyana, Tobago and Venezuela. *Phenacoccus herreni* is not present in Africa, however (Williams & Granara de Willink, 1992) (Figure 1.2).

Life cycle

The mealybugs are generally located on the underside of the cassava canopy leaves, mainly around major leaf veins (Figure 1.4) and at low density inside growing tips. With increasing density, they spread over the entire plant.

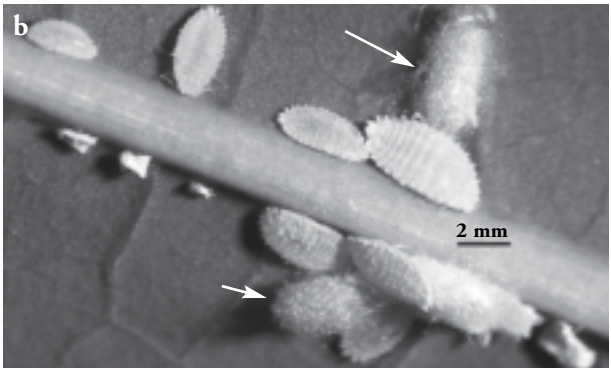
Phenacoccus manihoti has four developmental instars during its life cycle, producing only females (thelytokous parthenogenesis) (Figures 1.4a and 1.5). The adult female lays up to 500 eggs in an ovisac. The first instar is the most mobile stage (Nwanze, 1977) and is responsible for plant colonization within the same cultivated plot (Le Rü *et al.*, 1991). The entire life cycle from egg to adult takes about 21 days.

Phenacoccus herreni is bisexual, with a strong sexual dimorphism after the second instar (Figures 1.4b, c, d and 1.5). During the third and fourth instars, the males complete development to winged adults inside a cocoon. Similar to *P. manihoti*, adult females lay their eggs inside an ovisac. In this species, the first instar is also the most mobile stage (P.-A. Calatayud, unpublished observation). The life cycle between egg and adult is 20 days.

Species differentiation

Females of *P. manihoti* and *P. herreni* are similar in appearance. It is difficult to differentiate them because of the wide variation of morphological characters in both species. Differentiation of these two species can only be done by observations of live insects. *Phenacoccus manihoti* is pink and as mentioned above, reproduces by thelytokous parthenogenesis, whereas *P. herreni* is yellow and bisexual (Cox & Williams, 1981; Williams & Granara de Willink, 1992) (Figures 1.4 and 1.5). The question has been raised by Cox and Williams (1981) as to whether *P. manihoti* should not be considered the same species as *P. herreni*, differing only in the mode of reproduction. More modern tools used for molecular analysis have shown them to be genetically diverse, however.

B. Le Rü



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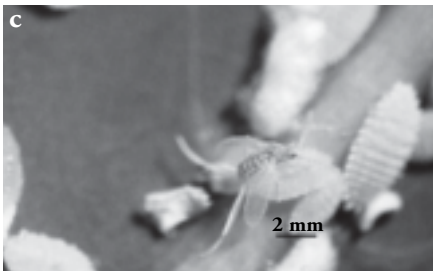

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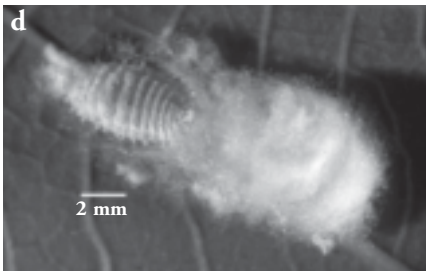


G. Guzman

Figure 1.4
Mealybug reproduction. In the case of *Phenacoccus manihoti*, only females are produced (a). In *Phenacoccus herreni*, the males develop inside a cocoon (b) and emerge as winged adults (c). Similarly to *P. manihoti*, the *P. herreni* adult female lays her eggs in an ovisac (d)

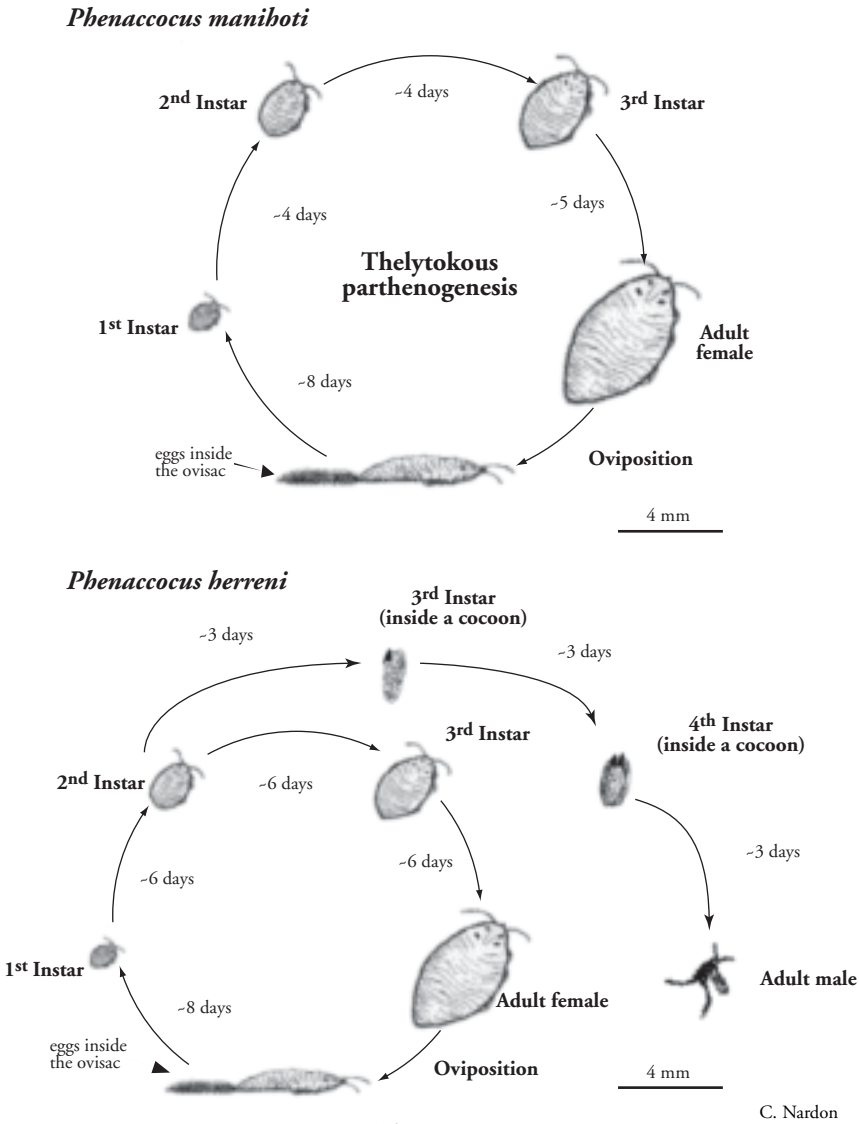


Figure 1.5
Developmental cycle of *Phenacoccus manihoti* according to Nwanze (1977) and *Phenacoccus herreni* according to Varela & Bellotti (1981)

RAPD (random amplified polymorphic DNA) analysis using the operon primers H9 and H16 has been used by Calvert and co-workers (L.A. Calvert *et al.*, unpublished) to test whether *P. manihoti* and *P. herreni* are separate species (see Appendix 1 for Materials & Methods) using populations of *P. manihoti* collected from San Lorenzo, Paraguay and Pointe Noire, Republic of Congo. The *P. herreni* populations were collected from Cruz das Almas, Brazil, and from the colony established from individuals collected at CIAT, Palmira, Colombia. As a positive control for species differentiation, adult females of *Phenacoccus madeirensis* from cassava plants grown in the CIAT fields were used. All the mealybug populations were collected from *M. esculenta*.

Among the *P. herreni* populations, the amplified products display similar electrophoretic patterns (Figure 1.6). For this species, there is a typical predominant PCR (polymerase chain reaction) fragment of ~250 bp obtained using the primer H9 and a doublet of ~1,600 bp using the primer H16 (arrows in Figure 1.6). On the other hand, *P. manihoti* populations show a distinct predominant PCR fragment of ~1,300 bp using the primer H9 and an ~800 bp fragment using the primer H16. The patterns of the amplified products of *P. madeirensis* for both sets of primers are very distinct from the other mealybug species tested. Analysis of *P. madeirensis* shows predominant PCR fragments of ~1,500 bp and ~1,300 bp using the primer H9 and H16, respectively. Both of these primers are useful for making a clear distinction among *P. herreni*, *P. manihoti* and *P. madeirensis*.

The use of these primers results in relatively few amplified products, most likely due to the fact that they are three distinct species which are evolutionarily different at the molecular level. Despite the geographic isolation of the *P. herreni* populations, both primers proved useful in also confirming that the two populations from Colombia and Brazil are the same species. The same conclusion could be made for the populations of *P. manihoti* from Paraguay and the Democratic Republic of the Congo (DRC), a result that is consistent with the historical accidental introduction of *P. manihoti* from South America to Africa. Since it is not easy to distinguish between *P. herreni* and *P. manihoti* females using morphological characteristics, the use of RAPD is a useful tool in differentiating the two species, especially in areas of Brazil such as Sao Paulo State, where they have overlapping ranges (A.C. Bellotti, CIAT, personal communication).

To confirm the placement of *P. herreni* and *P. manihoti* as separate species, molecular analysis comparing the mitochondrial 16S rDNA fragment was done by Cuervo and co-workers (2001). Such analyses have been used also to study phylogenetic relationships in whiteflies (Frohlich *et al.*, 1999; Calvert *et al.*, 2001) and in mealybugs

(Beuning *et al*., 1999; Downie & Gullan, 2004). Cuervo and colleagues (2001) confirmed the placement of *P. herreni* and *P. manihoti* in separate species and showed that the African and Latin American populations of *P. manihoti* are closely related. In addition, other evidence that the two forms are separate species was obtained from experiments showing the inability of the parasitoids of *P. herreni* to develop on *P. manihoti* (Cox & Williams, 1981; F. Schulthess, ICIPE, personal communication).

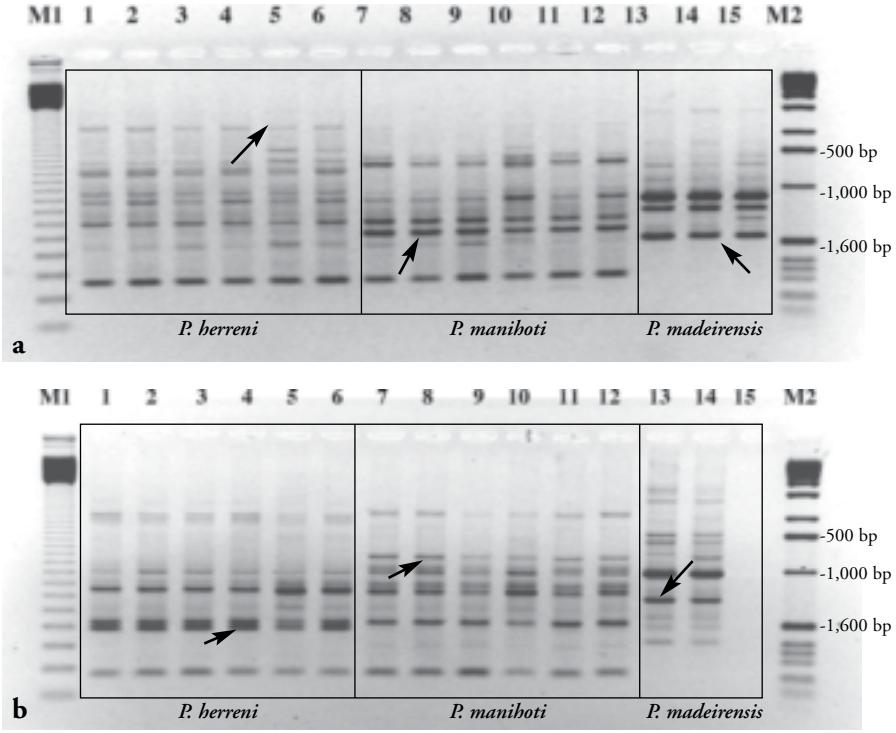


Figure 1.6
Agarose gel electrophoresis of the DNA products following RAPD of *Phenacoccus* mealybug genomic DNA by using the primers operon H9 (a) and H16 (b). In lanes 1-3, *P. herreni* from Colombia; lanes 4-6, *P. herreni* from Brazil; lanes 7-9, *P. manihoti* from Congo; lanes 10-12, *P. manihoti* from Paraguay; lanes 13-15, *P. madeirensis* from Colombia; M1: 123bp DNA ladder; M2: 1kb DNA ladder (BRL). bp: basepair; kb: kilobase

Reproductive biology

Attempts to induce parthenogenesis in *P. herreni* by keeping females in the absence of males have not been successful, but females of *P. manihoti* reared in the presence of males of *P. herreni* continue to reproduce parthenogenetically (Cox & Williams, 1981; P.-A. Calatayud, unpublished observation). The difference in reproductive mode between *P. manihoti* and *P. herreni* could be due to contamination of the parthenogenetic species with *Wolbachia*. From the literature, *Wolbachia*, a rickettsia-like organism, is reportedly widespread in invertebrates. It is estimated to infest 15-20% of all insect species (Werren, 1997), and has been found also in Crustacea, Arachnida and Nematoda. Transmitted vertically through the egg cytoplasm, it is able to undergo extensive intertaxon transmission. Examples of transmission between different orders of insects are reported (see Werren, 1997 for review). In arthropods, *Wolbachia* is known to alter host reproduction in four ways: through cytoplasmic incompatibility (O'Neil & Karr, 1990), parthenogenesis (Stouthamer *et al.*, 1993), feminization of genetic males (Rigaud *et al.*, 1991), and male-killing (Hurst *et al.*, 1999; Jiggins *et al.*, 2000).

Molecular analysis was used to test whether the *P. manihoti* used in the RAPD analyses were contaminated by *Wolbachia* (Calvert *et al.,* unpublished). All the aforementioned *P. manihoti* and *P. herreni* populations were tested by PCR for the presence of *Wolbachia* (see Appendix 1 for Materials & Methods). Two repetitions based on two independent DNA extractions were done on each strain in parallel with a positive (infected) control of *Trichogramma*. Based on the presence of a PCR fragment of 867 kb, indicative of *Wolbachia* contamination, all strains appeared to be negative for *Wolbachia* when using the classical PCR method. Long PCR demonstrated that the *P. herreni* from Brazil was only *Wolbachia*-infected strain (Figure 1.7).

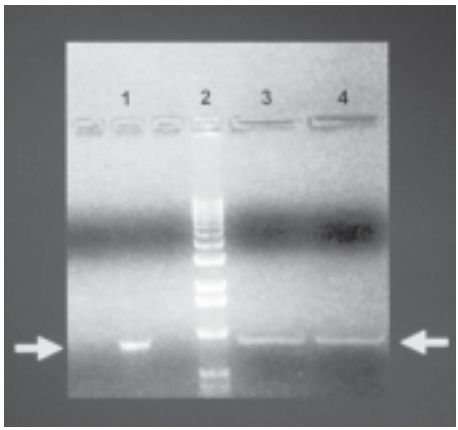


Figure 1.7
Agarose gel electrophoresis of the DNA products following long PCR amplification of *Phenacoccus* mealybug genomic DNA using specific primers for *Wolbachia* detection. Lane 1: positive control from *Trichogramma*; Lane 2: 1kb DNA Ladder; Lanes 3 and 4: *P. herreni* from Brazilian populations. Arrows show the DNA fragment at ~867 kb indicating the presence of *Wolbachia*

The PCR methods (classical and long) did not show evidence of *Wolbachia* in the other strains analysed (*P. manihoti* from Republic of Congo and Paraguay, and *P. herreni* from Colombia).

It is noteworthy that the *ftsZ* band intensity (*i.e.* from the specific *Wolbachia ftsZ* gene primers) from the *P. herreni*-Brazil sample was very weak compared to the *Trichogramma*-positive control, suggesting a low *Wolbachia* density within this strain. This indicates either a possible incompatibility between *Phenacoccus* and *Wolbachia,* or the possibility that *Wolbachia* has only recently colonized this mealybug population. Another possible explanation for the low detection level of *Wolbachia* in *Phenacoccus* could be that the *Wolbachia* strain used is only distantly related to the strain found in the *Drosophila melanogaster* from which the specific primers were derived. The results indicate clearly that *P. manihoti* parthenogenesis is not due to *Wolbachia* contamination.

2 Host-Plant selection

Phenacoccus manihoti and *P. herreni* are oligophagous insects which mainly colonize *Manihot* species. In Africa, limited infestation of *Talinum triangularae* Jack. (Portulacaceae) by *P. manihoti* also has been reported (Neuenschwander *et al.*, 1986). In South America, however, *P. manihoti* has been found on *Citrus* spp. (Rutaceae) and soybean, *Glycine max* (L.) Merr. (Fabaceae), while *P. herreni* has been reported only on *M. esculenta* (Williams & Granara de Willink, 1992). Under laboratory conditions, *P. manihoti* can be reared on poinsettia, *Euphorbia pulcherrina* Wild. (Euphorbiaceae)(Boussienguet, 1984).

The different resistance components of cassava to *P. manihoti* have been studied by Tertuliano and colleagues (1993). These authors identified varieties of cassava with different degrees of antixenosis or non-preference to the pest, *i.e.* cassava varieties more or less preferred by the insect (see also Chapter 5). This type of resistance generally occurs during initial plant selection and involves the physical and chemical characteristics of the plant. To further explain how host plants are identified by the insect, the types of sensory information mealybugs can detect using either the antennae or the labium were first described by Le Rü and colleagues (1995a and b). To determine the role of plant surface characteristics in plant selection, the probing behaviour on the surface and on the outer tissues of the plant by the mealybug was analysed (Renard *et al.,* 1998; Renard, 1999). Studies on the feeding behaviour have helped to determine the final decision made by the insect in plant selection (Calatayud *et al.*, 1994a; 2001a).

SENSORY SYSTEM OF MEALYBUGS

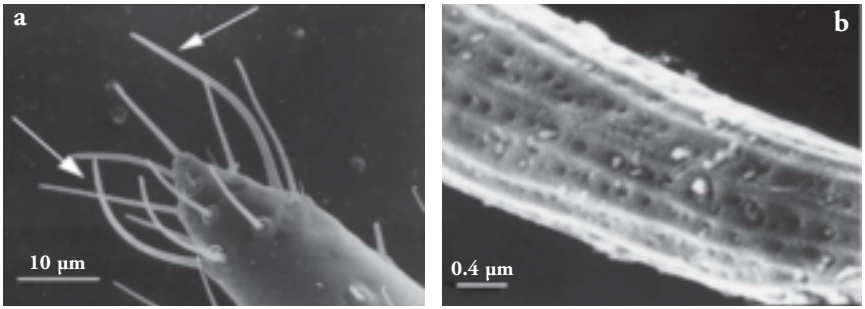
During plant surface exploration, the major sensory organs used by Sternorrhyncha are the antennal and labial sensilla. While the antennal sensilla of aphids and psyllids have been well studied, the labial sensilla have been poorly investigated, with only

three studies on aphids and whiteflies that show a high degree of anatomical variability among species (Backus, 1988; Walker & Gordh, 1989). In the Pseudococcidae, the external morphology of the antennal receptors has been examined by light microscopy only. A study on the citrus mealybug, *Planococcus citri* Risso (Pseudococcidae) has shown the presence of one and two basiconic sensilla with olfactory function on the subapical and apical segments of the antenna, respectively (Salama, 1971). Koteja (1980) studied the external morphology and the distribution of sensory receptors on the antennae of a few species of Pseudococcidae by light microscopy. In *Phenacoccus aceris* Geoffroy, he observed the presence of basiconic sensilla and sensilla chaetica (thin-walled pegs) with putative olfactory function on the last antennal segment. More recently, Le Rü and co-workers (1995a, b) investigated the external morphology of both antennal and labial sensilla of *P. manihoti* by scanning electron microscopy and their internal ultrastructure by transmission electron microscopy.

Antennae of *P. manihoti*

As in most of the Sternorrhyncha studied to date (Backus, 1988), the antennae of *P. manihoti* are equipped with a small number of sensilla (Figure 2.1). Its sensory system appears to be well diversified, however. Trichoid sensilla, distributed on all segments of the antenna and innervated by a single mechanoreceptive dendrite, have the characteristics of exteroceptors. They are located mainly on the distal part of antennal segments and it is thought that they may be involved in the mealybug's sense of touch. Their numbers are similar to those previously reported in other Sternorrhyncha, such as the Aphididae and Psyllidae (Backus, 1988).

A campaniform sensillum located on the pedicel and one basiconic sensillum on the flagellum were also detected on the antenna of *P. manihoti* (Le Rü *et al.*, 1995a). These sensilla, which have the characteristics of proprioceptors (Koteja, 1980), have been found in other mealybug species and also in Aleyrodidae, Aphididae, Psyllidae and Thysanoptera, among other orders (Koteja, 1980). Such sensilla are likely to help the insect in guiding the movement of its antennae (Bromley *et al.*, 1980). As previously described by Koteja (1980) for the antennae of many Coccinae species, *P. manihoti* antennae also possess coeloconic sensilla located ventrally on the pedicel and flagellum. The author did not attribute any function to those receptors, however, as his study was based on only external cuticular structures. Le Rü and colleagues (1995a) have shown that these sensilla are related to poreless sensilla with inflexible sockets, probably having thermo/hygroreceptor functions.



J. Le Lannic

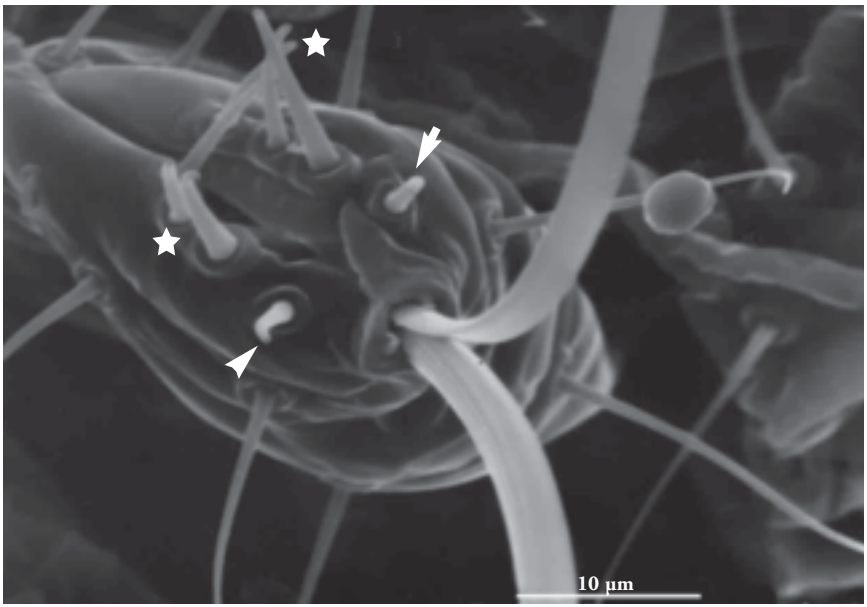
Figure 2.1
Antenna of 1st instar of *Phenacoccus manihoti*. Ventral view of the 9th antennal segment (a) showing the position of the different sensilla; the longest sensilla, which have a chemoreceptive function, are indicated by the arrows. Detail of one of these sensilla (b) showing a perforated cuticle with numerous pores (Le Rü *et al.*, 1995a)

Koteja (1980) reported the presence of several types of sensilla on the three distal segments of the flagellum in Pseudococcidae. This was confirmed for *P. manihoti* by Le Rü and co-workers (1995a), who reported five different types of sensilla. It was shown that these sensilla are related to uniporous chemosensilla and multiporous chemosensilla with probable gustatory and olfactory functions, respectively. Unlike the other families of Sternorrhyncha (Aphididae, Aleyrodidae and Psyllidae), the antennae of Pseudococcidae are not equipped with plate organs, whose olfactory function has been demonstrated in aphids by Bromley & Anderson (1982). In Pseudococcidae, the olfactory function appears to be mediated by peg sensilla localized on the apex of the antenna. Moreover, similar to Aphididae and Psyllidae, the gustatory function is also localized on the apex of the antenna in Pseudococcidae.

Labium of *P. manihoti*

In Sternorrhyncha, the sensory receptors on the labium have been poorly described compared to those on the antennae. However, it has been observed that the tip of the labium of the aphid *Brevicoryne brassicae* L. possesses only mechanoreceptors (Wensler, 1977; Tjallingii, 1978a), while in the whitefly *Parabemisia myricae* Kuwana, the labium is equipped with chemoreceptors (Walker & Gordh, 1989). The morphology and ultrastructure of sensilla present on the tip of the labium of Pseudococcidae were described

for the first time on *P. manihoti* by Le Rü *et al.* (1995b), who reported 10 pair of trichoid hairs with a probable mechanoreceptive function distributed over the labium (Figure 2.2). It has been suggested by Backus (1988) that such hairs may be involved in the identification of the physical characteristics of the plant surface while the insect is selecting the site of the probe, and may likely detect the depth and angle of the labium when the mouthparts or the stylets are inserted during feeding. On the labium of *P. manihoti*, six uniporous chemosensilla and two multiporous chemosensilla with likely gustatory and olfactory functions, respectively, have been recorded. Le Rü and colleagues (1995a) reported the presence of gustatory sensilla on the labium of Pseudococcidae, while Walker & Gordh (1989) described such sensilla on the labium of Aleyrodidae. The former authors reported for the first time the presence of olfactory sensilla in Sternorrhyncha; the presence of such receptors had been described previously only in Auchenorrhyncha and *Nilaparvata lugens* Stal (Delphacidae) by Foster *et al.* (1983).



C. Nardon

Figure 2.2
Labium of 4th instar larvae of *Phenacoccus manihoti*, showing six sensilla having contact chemoreceptor as well as mechanoreceptor functions (stars) and two sensilla having olfactory functions (arrows), according to Le Rü *et al.* (1995b)

In conclusion, as in most Sternorrhyncha studied to date, the apex of the antenna and the tip of the labium of *P. manihoti* are equipped with a small number of sensilla, generally characteristic of a high specificity in feeding (Chapman, 1982). The sensory system of *P. manihoti,* however, appears well diversified, with 58 sensilla of nine different types on the antenna and 30 sensilla of four different types on the labium. Together, the antennal and labial sensilla most probably mediate orientation to the cassava by crawlers (larvae) of *P. manihoti* and the initial stages of plant acceptance, while dabbing the plant surface with the ventral side of the last antennal segment and the tip of the labium.

HOST-PLANT RECOGNITION BY MEALYBUGS

Behaviour on plant surface

Video observations done on *P. manihoti* (Renard *et al.*, 1998; Renard, 1999) have shown that when selecting the fixation site, the mealybug alternates between walking and stopping (called the 'probing *sensus*' by Klingauf, 1970). When the mealybug walks, the antennae are pointed forward and the labium quickly taps the leaf surface (brief contact). When the insect stops, it rubs the surface with the tip of its forelegs and slowly taps it with the lower side of the last antennal segment and with the tip of the labium (more prolonged contact). The first stop may take place on the upper or lower face of the leaf (Figure 2.3). Prior to probing, simply walking on the leaf surface and tapping the leaf with the antennae and labium allows the insect to differentiate between the more- or less-preferred plants. On a preferred cassava variety such as 'Incoza', the passage on the lower leaf face is quickly followed by a nearly immediate fixation. On a less preferred cassava variety such as 'M'pembé', an increase in the duration of walks is observed. On a non-host plant such as *Talinum triangularae* (Portulacaceae), the walks are long (especially on the upper leaf face) and accompanied by numerous changes of side as the insect attempts to escape. This pattern of behaviour occurring on a less preferred plant and on a non-host plant is observed in many Sternorrhyncha (Backus, 1988), and especially in aphids (Ibbotson & Kennedy, 1959; Klinghauf, 1970) and whiteflies (Noldus *et al.,* 1986; Walker, 1987).

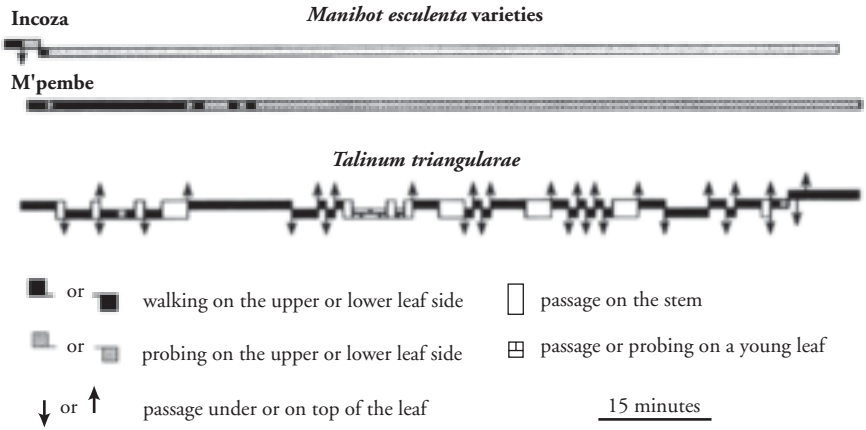


Figure 2.3
Characteristic ethograms (a succession of behavioural items observed over a time period) of the *Phenacoccus manihoti* testing behaviour observed over 90 min on leaves of two host plants (two cassava varieties) and one non-host plant (*Talinum triangularae*) (after Renard, 1999)

The test probing behaviour by the insect before feeding, constituting the first step in host-plant acceptance, has been studied in detail for *P. manihoti* (Renard *et al.*, 1998). To observe the relationship between the movement of mealybug organs (body, legs, antennae and labium) and the stylets' pathways inside the plant tissues, video observations of the mealybugs combined with electropenetrography (EPG), a technique to electrically follow the stylets' pathway, were used. *Phenacoccus manihoti* exhibits three phases of test probing (Renard *et al.*, 1998). During the first phase, the mealybug repeatedly drums and rubs the plant surface with the ventral part of the antennae, labium and foreleg tips. Thereafter, the antennae vibrate. At the end of this phase, the labium remains in contact with the plant surface without any stylet penetration into the plant tissues. According to Städler (1986), the insect probably evaluates the nutritional quality of the plant during this phase, the recognition having already taken place during this behavioural event. The repeated contacts of the olfactory and gustatory sensory organs of the mealybug, located principally on the antennae and labial tip, suggest that, as is hypothesized for aphids (Klingauf, 1971; Greenway *et al.,* 1978), the mealybug is able to perceive the odours present in the thin air layer above the leaf surface (the boundary layer).

In the second phase, the insect quickly moves its head up and down, moves the antennae forward and backward, and rubs the plant surface with its forelegs. During this phase, the stylets pass through the epidermal and inner tissues of the plant. Finally during the third phase, the mealybug becomes more agitated. It can be observed to stand up using its rear legs and push the upper part of its body against the plant. The stylets continue their progression into the tissues, which is principally extra- or intercellular (see also section on feeding behaviour, below), until they reach the phloem. Renard and colleagues (1998) observed that the three phases described above and the progression of the stylets in the leaf tissues are rendered difficult in less preferred cassava varieties. This last aspect has been confirmed in EPG studies by Calatayud and co-workers (1994a).

Influence of host-plant surface characteristics

Upon contact with the plant surface, the insect is able to obtain both chemical and physical information on plant quality by tactile (mechanosensory) and contact chemosensory (taste or gustation) stimuli (Schoonhoven *et al.*, 1998).

■ **Physical characteristics**:
The physical characteristics of the leaf surface may not play an important role in host-plant recognition. Renard (1999) has shown that for *P. manihoti*, for example, neither the trichome density nor the waxy thin layer of the lower leaf epidermis (the leaf surface colonized by mealybugs) have a relationship to preference for a cassava variety or the status of a plant as a non-host. Host-plant acceptance by *P. manihoti* therefore appears to be mainly determined by the chemical characteristics of the plant surface.

■ **Chemical characteristics**:
Mealybugs prefere to colonize *Manihot* species. Cassava has a high content of cyanide compounds in the leaves, stems and roots (Arihantana & Buckle, 1986; Ezeala & Okoro, 1986; Pancoro & Hughes, 1992). The predominant cyanogen is linamarin (Butler *et al.,* 1965) (Figure 2.4), a cyanogenic glucoside which is hydrolyzed into glucose and free hydrogen cyanide (HCN) after tissue damage (Conn, 1980). This hydrolysis is called 'cyanogenesis'.

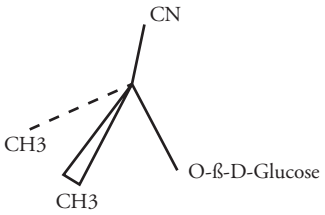


Figure 2.4
Linamarin structure according to Conn (1980)

Further experiments done on *P. manihoti* by Renard (1999) demonstrated that if cassava leaves are previously soaked in methanol, thus preferentially removing the polar compounds (including linamarin) from the leaf surface, the insects do not recognize the host leaves. Without solvent treatment, 80% of the insects were fixed on leaves after infestation *versus* only 30% after methanol treatment. When these methanolic extracts were applied to the Parafilm enclosing an artificial diet (see Chapter 3 for details of the set-up), an increase in the proportion of insects fixed on the diet rose from 10% in the control to 43% after treatment by the extracts. Analysis of the methanolic extracts by gas chromatography coupled with mass spectrometry (GC-MS) revealed the presence of linamarin at a concentration of 1–4 ng/cm^2 (Renard, 1999). To confirm the role of linamarin in host-plant recognition by the insect, commercial linamarin (620 µg/L) was deposited on Parafilm enclosing the artificial diet. The proportion of insects fixed on the diet increased from 33% in the control to 93% after treatment (Renard, 1999).

These results all indicate that linamarin, present on the phylloplane of cassava, induces the host-plant recognition behaviour of *P. manihoti*. Even at the very low linamarin concentration of only a few ng/cm^2, this compound could be detected by *P. manihoti* larvae because of the high sensitivity of the sensory organs found generally in insects. Moreover, the repeated contacts of the olfactory and gustatory sensory organs, located principally on the antennae and labial tip of *P. manihoti* (Le Rü *et al.*, 1995 a, b), enable the insect to perceive compounds such as the linamarin present in the thin air layer of the phylloplane. These repeated contacts of the sensory organs with the plant serve likely to increase the amount of sensory input to the central nervous system.

Primary compounds such as sucrose and free amino acids also are found on the cassava leaf surface. Nevertheless, there is no evidence that these compounds play a crucial role in host-plant recognition by *P. manihoti* before probing (Renard, 1999). Additional analyses of cassava leaf surface compounds reveal also the presence of triterpenoids, likely including ß-amyrin (Renard, 1999). These compounds, frequently present on the plant phylloplane (Wollenweber *et al.*, 1999), are known to be repulsive to many insect species (see review by Eigenbrode & Espelie, 1995), however their effect on the cassava mealybugs has not been studied.

In conclusion, linamarin, a cyanogenic compound present on cassava phylloplane and characteristic of *M. esculenta*, the host-plant of *P. manihoti* and *P. herreni*, appears the most plausible chemical involved in host plant recognition and acceptance by the insects. This has been clearly demonstrated by Renard (1999) in the mobile stages (crawlers) of *P. manihoti,* the stage most involved in plant colonization.

Feeding behaviour

By virtue of possessing stylets, the mealybugs have a sap-sucking mode of feeding. The mouthparts of the mealybugs, and in Sternorhyncha in general, are composed of four stylets (two mandibularies and two maxillaries)(Grassé, 1951) (Figure 2.5). In the Coccidae and Pseudococcidae, the stylets are folded into a loop inside the crumena in the labium. The total length of the stylets of *P. manihoti* is estimated to be about 870-900 µm.

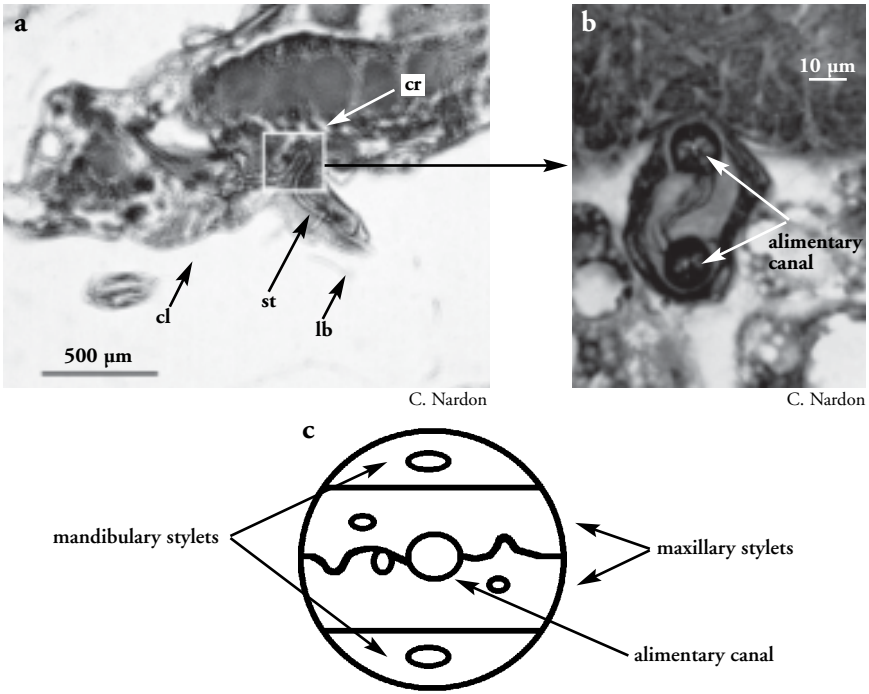
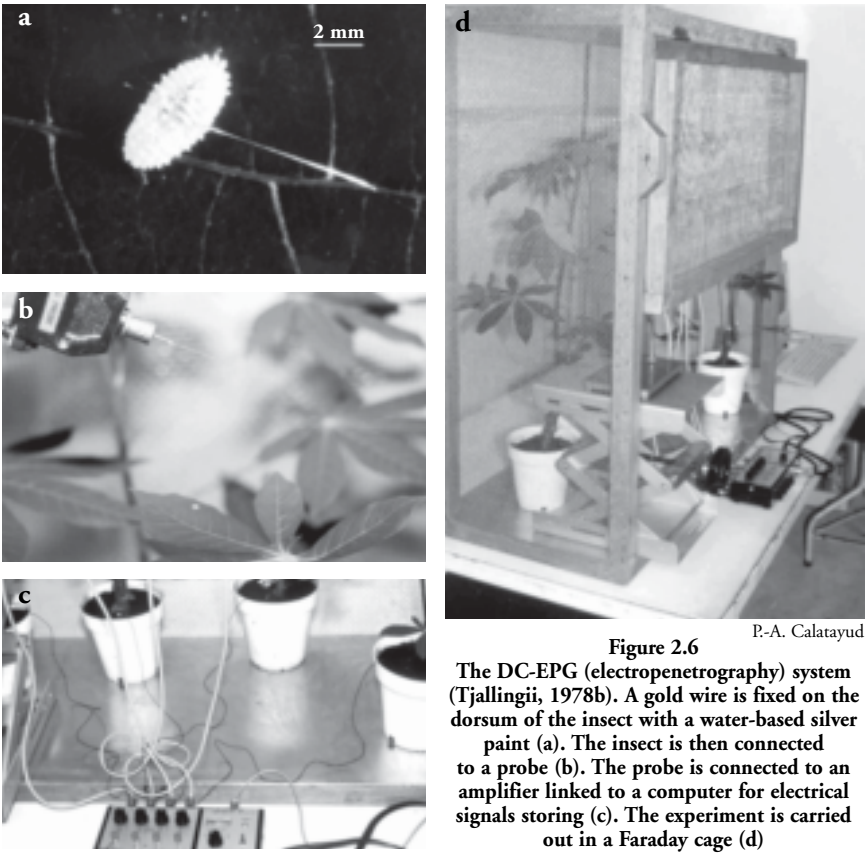


Figure 2.5
Anatomical relationships of the labium (lb) and the stylets (st) in *Phenacoccus manihoti* (cl, clypeus; cr, crumena) observed in a longitudinal section of the body (a). Transverse section of the stylets folded up into a loop inside the crumena (b). The diameter of the alimentary canal is estimated at about 2 µm. Schematic view of the stylets in Sternorhyncha according to Grassé (1951) (c)



P.-A. Calatayud

Figure 2.6
The DC-EPG (electropenetrography) system (Tjallingii, 1978b). A gold wire is fixed on the dorsum of the insect with a water-based silver paint (a). The insect is then connected to a probe (b). The probe is connected to an amplifier linked to a computer for electrical signals storing (c). The experiment is carried out in a Faraday cage (d)

The feeding behaviour of the Coccidae and Pseudococcidae has been described by several authors (Pesson, 1944; Albrigo & Brooks, 1977; Campbell, 1990; Molyneux *et al*., 1990), all of whom report a phloemophagous (phloem-sap feeding) behaviour. An assessment of the pathways followed by the stylets through the leaf tissues is now possible using electropenetrography (EPG) (Tjallingii, 1978b) (Figure 2.6). This technique, developed on aphids, allows direct electrical monitoring of the stylets' pathway inside the plant tissues and was used for the first time on mealybug species (particularly *P. manihoti* and *P. herreni*) by Calatayud and co-workers (1994a; 2001a). Similarities in the EPGs from mealybugs and those of aphids and whiteflies

(Tjallingii, 1978b; Janssen *et al.*, 1989) allowed adoption of a standard labelling pattern defined by Tjallingii (1988). The EPG data from aphids has been used as a reference for correlating the EPG patterns with various components of the stylet penetration process (see Figure 2.7 for interpretation of EPG patterns).

The EPG recordings show clearly the phloemophagous feeding characteristic of mealybugs. How these insects locate the sieve tubes is still not clearly understood, however. The guiding factors are probably a combination of physical and chemical stimuli (Rahbé *et al.*, 2000). Mealybugs seem to display an exclusive extracellular route to the phloem, with periodic intracellular punctures identified by drops in the electrical potential (Figure 2.7). Phases resembling xylem ingestion are occasionally observed.

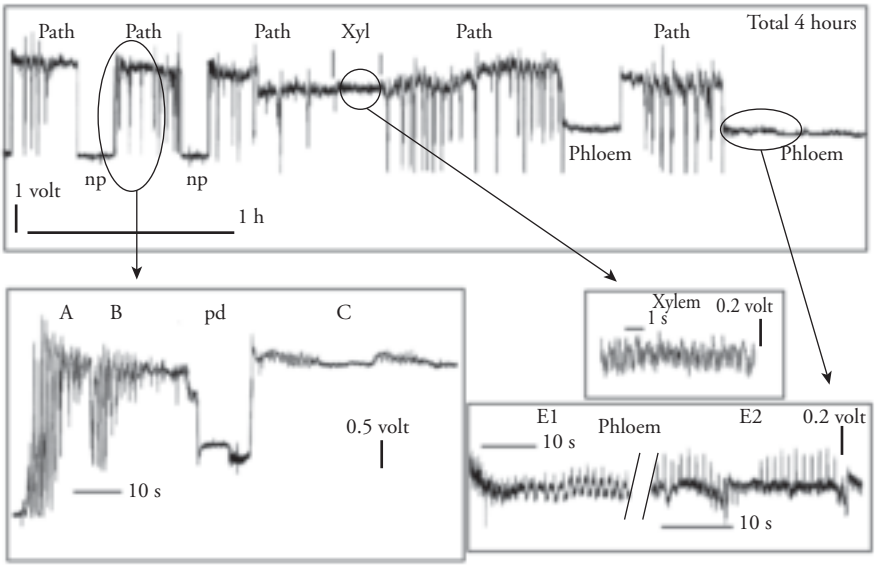


Figure 2.7
Typical EPG (electropenetrograph) patterns registered with *Phenacoccus manihoti* and *P. herreni* using the DC-EPG system of Tjallingii (1988). Model of 4 hours' recording, showing among the paths sequences of contact of mouthparts with leaf tissues (A and B, beginning of penetration showing big waves of salivation); cell-wall activities (C, showing small waves of salivation); cell punctures (pd, potential drop due to crossing of the membrane cell and its transmembrane potential); xylem sap ingestion (Xyl—no potential drop is registered because the xylem cells do not possess a cell membrane); and phloem cell ingestion, beginning with a potential drop due to the puncture of the phloem cell membrane and showing two sub-patterns (E1 and E2) of peaks of sap ingestion (Calatayud *et al.*,1994a and 2001a). np: non-penetration, *i.e.* stylets outside the leaf tissues

A comparison of EPGs from mealybugs (*P. manihoti* and *P. herreni*) with those from aphids shows some differences that could be significant in distinguishing their interactions with the host plant. As observed by Calatayud and colleagues (1994a), the main differences include the following: first, the cell puncture (pd pattern in Figure 2.7) duration is higher (about 10–20 s) in mealybugs, including two other Pseudoccoccidea species (*Ferrisia virgata* Cock. and *Rastrococcus invadens* Williams), while it is only about 5–7 s in aphids. Secondly, the number of cell punctures before reaching the phloem sap is much lower in mealybugs (10–20/h) on all plants than in aphids (50–60/h). Lastly, the minimal time to reach the phloem is much higher in mealybugs, at 1.5 h on a favourable host and 2.8 h on an unfavourable host for *P. manihoti*, while it is often less than 15 min for aphids. These differences emphasize the lower mobility of mealybugs within their feeding sequence as compared to aphids.

Phenacoccus herreni males feed only on the phloem sap of the host plant until the second instar; while in the cocoon, they do not feed until they emerge as winged adults. The females, on the other hand, feed on phloem sap throughout their life cycle, indicating that they cause more damage to the cassava plant than males (Polania *et al.*, 1999).

- **Influence of plant species**: A comparison of the behaviour of *P. manihoti* on different plant species of varying preference reveals significant correlations between some EPG parameters and the host status or the plant's susceptibility index (Calatayud *et al.*, 1994a). Host status appears to be linked to phloem accessibility, suggesting that early plant rejection due to delays in phloem-finding may result in antixenosis.

- **Influence of location on leaf**: It has been demonstrated that in the case of *P. herreni*, the insect's location on a leaf strongly influences the EPG parameters, and thus its feeding behaviour (Calatayud *et al.*, 2001a). Studies have shown that feeding near a major leaf vein—the general location of cassava mealybugs on leaves—facilitates phloem-finding behaviour. In conclusion, it is clear that pre-phloem interactions (mainly the intercellular pathways of the stylets) are the most important for host-plant acceptance or feeding site location.

- **Influence of plant chemistry**: To investigate the links between the feeding behaviour of *P. manihoti* and the leaf chemistry of the host plant, chemical analysis of cassava leaves was performed by Calatayud and co-workers (1994a). Some secondary compounds were found in leaf apoplastic (intercellular) fluids, but none of the

cassava varieties tested, including wild cassava species such as *M. cecropiafolia*, *M. violaceae* and the hybrid *M. esculenta* x *M. glaziovii,* showed detectable amounts of alkaloids (P.-A. Calatayud, unpublished data).

Cyanogenic compounds have been found to be restricted to true hosts (cassava). Cyanogenic compounds in the bound form of glucosides, mainly linamarin, are generally present in the vacuolar compartment of cassava meristematic tissues (Conn, 1980). When correlating cyanide content with EPG parameters, the best correlation has been observed with the duration of intracellular punctures (Calatayud *et al.*, 1994a), indicating a possible use of cyanogenic compounds as allelochemicals for host recognition during the cell penetration process by *P. manihoti*.

No correlation has been shown between flavonoid levels and any of the individual probing parameters, nor with the susceptibility index (*cf* antixenosis), indicating that the flavonoids are not involved in the initial interaction between the mealybug and its host. On the other hand, the level of phenolic acids in the leaves has been shown to be strongly correlated to the EPG parameters, and is associated with a delay in reaching the phloem vessels (Calatayud *et al.*, 1994a). On the less preferred cassava variety with a high phenolic acid level in its extracellular fluids, the mealybugs spent the longest time in searching for the phloem. This is interesting in view of the role of phenolic acids in cell wall structures and as precursors of lignins, cutins/suberins and phenolic-coupled pectins, all of which could interact with the oxidizing enzymes found in the saliva of such phloemophagous insects (Fry, 1983; Goodman, 1986).

3 Nutritional Physiology

As reported in Chapter 2 on host selection, *P. manihoti* and *P. herreni* possess mouthparts with four stylets and show a phloem-feeding behaviour with a predominance of extracellular or intercellular pathways of the stylets. The role of the saliva in such insects is therefore important, since it is thought to be a carrier of enzymes and gustatory stimuli to help the stylets penetrate the plant tissues *via* formation of a kind of tunnel (Miles, 1972). The role of digestive enzymes is also important in the nutritional physiology of insects in general, as their physiology is related to the type of meal ingested.

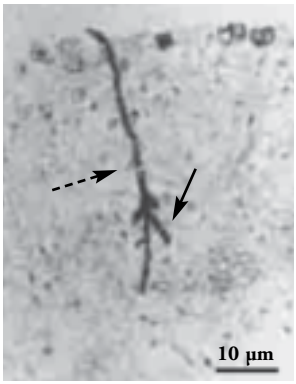
The first strategy in evaluating the nutritional requirements of an insect is to analyse the biochemical constituents of its ingesta. Consequently, for the cassava mealybugs (phloem-feeding insects), biochemical analysis of the constituents of phloem sap is important. After analysing the ingesta constituents, the next step is to rear the insect on a defined artificial diet of known constituency (a holidic diet). Since the early 1960s, the use of such diets has provided important information on the nutritional requirements of phloem-feeders, mostly aphids (Auclair, 1965; Mittler, 1970; Dreyer & Jones, 1981; Febvay *et al.*, 1988), but also the mealybug *Planococcus citri* Risso (Gothilf & Beck, 1966). Artificial diets have proven useful also in plant resistance studies, in testing for the presence of plant resistance factors (Dreyer & Jones, 1981; Rahbé *et al.*, 1988; van Helden *et al.*, 1995; Chen *et al.*, 1996).

The capacity to utilize simple food constituents such as those found in the phloem sap of plants depends in such insects on the presence of symbiotic microbes or endosymbionts. Pseudococcids are associated with endosymbionts (Buchner, 1965; Tremblay, 1989). As is the case in aphids (Srivastava, 1987), endosymbionts should aid in the synthesis of the mealybug nutritional requisites that plants do not provide at all or provide in insufficient quantities (*e.g.* some protein amino acids).

SALIVA

Cassava mealybugs display a typical extracellular penetration behaviour, with secretion of a branched salivary sheath (of which only one branch contains the actual stylets) that

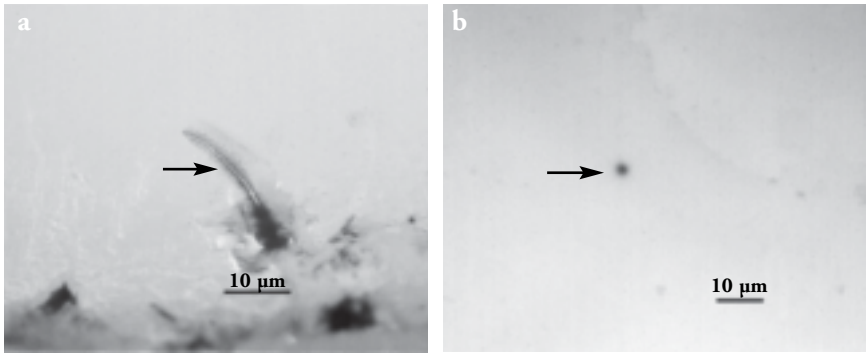
induces little damage and probably leads in most cases to a phloem vessel (Figure 3.1). The saliva should therefore contain mostly enzymes involved in the degradation of the plant cell-wall constituents, such as those degrading pectin and cellulose.



C. Nardon

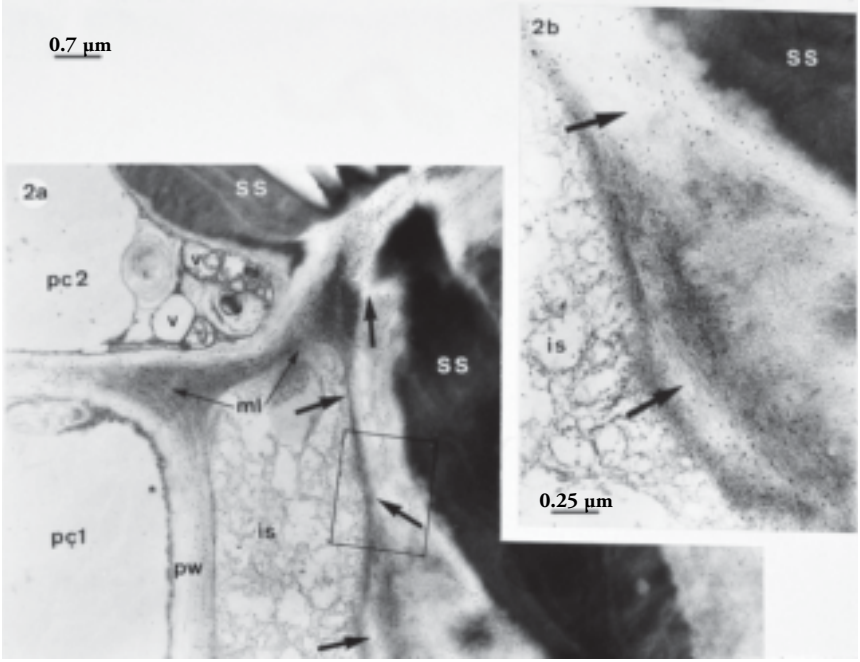
Figure 3.1
Tranverse section of a cassava stem showing a branched stylet sheath left by *Phenacoccus manihoti* after feeding (arrow). Note the cell puncture gap in the progress of the sheath (dotted arrow)

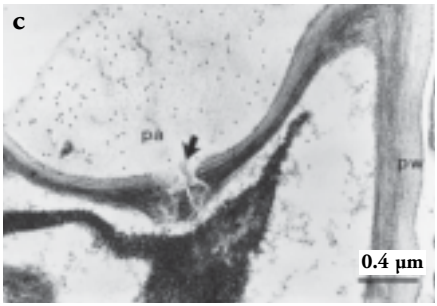
Using the method developed by Ma and colleagues (1990), it has been possible to visualize in pectin-agarose gels the presence of enzymes degradating pectin, such as the pectinesterase from salivary secretions of living *P. manihoti* (Calatayud, 1993) (Figure 3.2). Further cytochemical studies have revealed that the degradation of the middle lamellae (the middle part of the cell wall) and the primary cell wall is associated with mealybug pectinesterase found in salivary secretions (Calatayud *et al.,* 1996) (Figures 3.3a, b), thus confirming the involvement of this enzyme in the stylets' extracellular pathway. In summary, during probing, mealybugs secrete pectinolytic enzymes involved in the degradation of the cassava cell wall, thereby facilitating penetration of the stylets into the host tissues.



P.-A. Calatayud

Figure 3.2
Specific activity of pectinesterase in stylet sheaths (arrows) left by a living *Phenacoccus manihoti* mealybug feeding in pectin-agarose gel plates: (a) transverse view, and (b) overview of the gel plate. A dark red-stained halo, showing pectinesterase activity around the site of a stylet sheath left by *P. manihoti,* can be seen in (b)





B. Boher

Figure 3.3
Micrographs of mealybug-infested cassava leaf phloem tissues fixed in glutaraldehyde/osmium tetroxide according to Calatayud *et al.* (1996). A transverse section of an infested leaf was treated with anti-pectin antibodies stained by gold particles (a), resulting in the labelling of the intercellular space (is). Portions of the middle lamella (ml) and primary wall (pw) of phloem cells (pc 1, 2 and 3) adjacent to the insect salivary sheath (ss) show an uneven labelling (see arrows). No gold particles are seen over the sheath in (b), a zoom view of (a). In (c), a papilla occludes a plasmodesmatal area (pa, arrowhead) of a phloem cell in the insect feeding zone. The papilla appears evenly labelled after treatment with anti-beta (1,3)-D-glucopyranose polyclonal antibodies stained by gold particles. pw, primary wall; v, vacuole

Although the stylet sheaths left in plants by feeding mealybugs induce little mechanical damage to host tissues, they may be toxic to the plant, however, due to the presence of remaining pectic enzymes. Moreover, the characteristic leaf curling caused by mealybug infestation (see Chapter 1) might be the result of calcium extraction linked to pectic substances in the cell walls of cassava leaves, probably after pectin degradation by salivary pectinesterase, as postulated by Vargas *et al.* (1989) for *P. herreni*. The EPG recordings (see Chapter 2) have revealed that mealybugs can ingest apoplastic fluid (Calatayud *et al.*, 1994a; 2001a) and thus probably also the intracellular calcium which is involved in cell wall rigidity. However, no rupture of the cellulose layer in the cell wall after stylet penetration was evidenced, indicating the absence of salivary cellulases in mealybugs (Calatayud *et al.*, 1996). This is not surprising, as such salivary enzymes are reportedly absent in other Sternorrhyncha, as for a number of aphid species (Miles, 1988).

The use of cytochemical techniques allows an understanding of the plant's reaction to the rupture of its cell walls (Calatayud *et al.*, 1996). This approach has revealed that callus deposits are associated with damage caused by stylet penetration within the phloem cells (Figure 3.3c). It is plausible that callus formation is involved in the resistance mechanisms of cassava to *P. manihoti* infestation. Such a reaction is also thought to contribute to cassava resistance to *Xanthomonas campestris* pv. *manihotis* (Boher *et al.*, 1995) and to plant resistance to fungal infection (Kovats *et al.*, 1991). Formation of callus deposits therefore cannot be solely considered as an efficient plant defense mechanism against mealybug infestation, but rather is likely a non-specific plant reaction to phloem vessel healing which can be easily by-passed by the insect's stylet pathway (P.-A. Calatayud, unpublished observations).

DIGESTIVE ENZYMES

Insects feeding on phloem sap—a diet of simple food constituents such as free amino acids and sugars— do not possess a complex digestive system, but one constituted of only a midgut on which is attached an excretory organ (Malpighian tubules) (Figure 3.4) (Pesson, 1944; Grassé, 1951). Interestingly, the mealybug digestive system has also a filter chamber which is generally absent in aphids (Grassé, 1951), but which plays an important role in regulating the osmotic pressure and accumulation of nutrients (Yavada & Chandel, 1969). This is formed by a close connection of the two midgut tips folding up into itself (Pesson, 1944). Such a structure is particularly important for insects that ingest a large volume of phloem sap.

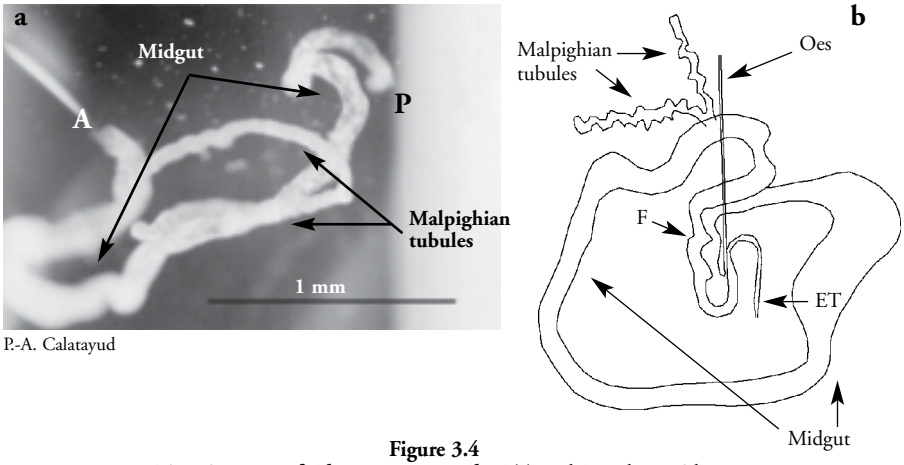


Figure 3.4
Digestive tract of *Phenacoccus manihoti* (a) and Pseudococcidae in general, according to Pesson (1944). In (b) are shown the A, anterior part of the midgut; P, posterior part of the midgut; Oes, oesophagus; ET, excretory tube; F, filter chamber—the contact of the two midgut tips.

Since a significant proportion of the ingested sap nutrients are excreted, it is likely that the insect will have an intestinal pH in a range similar to that of the ingested diet. The pH of the phloem sap reported for many plant species is slightly to moderately alkaline (pH 7.2–8.5)(Ziegler, 1975). Moreover, phloem-feeders respond to the pH of their diet, preferring diets with a slightly alkaline pH; for example, both *P. herreni* and *P. manihoti* can be raised on an artificial diet at pH 7.5 (Gothilf & Beck, 1966; Calatayud *et al.*, 1998; Calatayud, 2000; Calatayud *et al.*, 2002a). By using pH indicators, the pH of the midgut of *P. herreni* has been estimated as being slightly alkaline at pH 6.8–7.6 (D.F. Mùnera *et al.*, unpublished data) (see Appendix 2 for Materials & Methods).

A rapid semi-quantitative analysis of the enzymatic activities of the midgut of *P. herreni* after feeding was performed by D.F. Mùnera and co-workers (unpublished) using an API-ZYM system (see Appendix 2 for Materials & Methods). By comparing the enzymatic activities in the midgut with those identified in the body after midgut extraction, it was found that the major activities exhibited by the whole midgut were for alkaline phosphatase, esterase (C4) and leucine aminopeptidase (Figure 3.5).

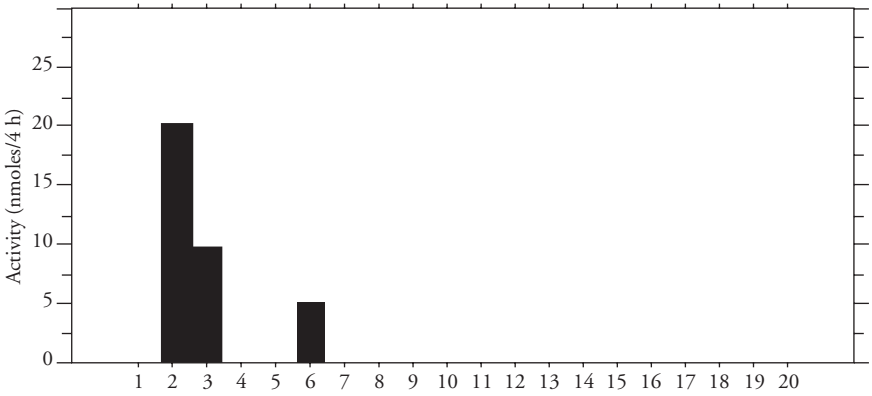
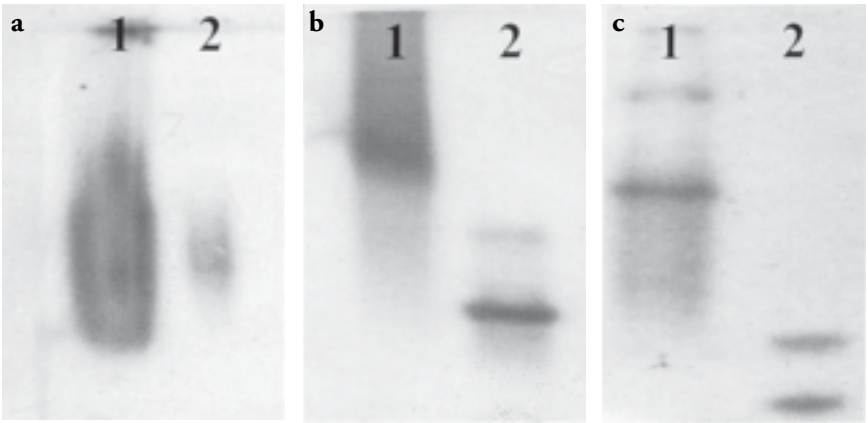


Figure 3.5
Semi-quantitative analysis of enzyme activities of the midgut of *Phenacoccus herreni* after feeding. Enzyme activities correspond to the activities found in the dissected midgut alone. Activities correspond to the release of 5, 10 and 20 nmoles of substrate per 4 h of incubation at 37°C (rate 0: activity not detected). Enzymes tested: Lanes (1) control, (2) alkaline phosphatase, (3) esterase (C4), (4) esterase lipase (C8), (5) lipase (C14), (6) leucine aminopeptidase, (7) valine aminopeptidase, (8) cystine aminopeptidase, (9) trypsin, (10) alpha-chymotrypsin, (11) acid phosphatase, (12) naphthol-AS-BI-phosphohydrolase, (13) alpha-galactosidase, (14) beta-galactosidase, (15) beta-glucuronidase, (16) alpha-glucosidase, (17) beta-glucosidase, (18) *N*-acetyl-beta-glucosaminidase, (19) alpha-mannosidase, (20) alpha-fucosidase

The presence of the three major intestinal enzymes revealed by the API-ZYM system was confirmed by the development of protein zymograms (Figure 3.6). A single major band was observed for alkaline phosphatase, whereas two bands of activity were evident for esterase and leucine aminopeptidase.

Mealybugs feed on diets constituted of simple food constituents, hence only a few digestive enzymes are detected. Of the 19 common enzymes analysed using the API-ZYM system (Figure 3.5), only three were evident in the digestive tracts of *P. herreni*. The enzyme alpha-glucosidase (or invertase) which hydrolyzes sucrose was not detectable in *P. herreni*. Since a significant amount of sucrose is excreted by such insects and is generally found in large proportions in the honeydew, sucrose (the major compound of phloem sap) is thought to be more of a phagostimulant than a nutrient.



D.F. Mùnera

Figure 3.6
Zymograms of alkaline phosphatase (a), esterase (b) and leucine aminopeptidase (c) present in the midgut (Lanes 2) of *P. herreni* (Lanes 1, positive controls). Zymograms were run on a 10-15% gradient native polyacrylamide gel; a western blot was carried out and then stained. For zymograms (b) and (c), the bands of activities have different profiles because the enzymes revealed in the midgut do not have the same weight and structure as the pure enzymes used as positive controls. However, all bands visualized in the samples were actually due to the enzymatic reactions tested

No significant levels of protease activity (trypsin- or chymotrypsin-like activity) were detected in the midgut of *P. herreni*. However, there was evidence of a peptidase enzyme (leucine aminopeptidase) (Figures 3.5 and 3.6). The absence of protease in the gut of phloem feeders was earlier observed in *Acyrthosiphum pisum* (Harris) (Rahbé *et al.*, 1995) and aphids in general (Auclair, 1963; Srivastava, 1987). Thus, not surprisingly, most protease inhibitors and especially trypsin inhibitors, are inactive in such insects, as was seen in *A. pisum* (Rahbé & Febvay, 1993; Rahbé *et al.*, 1995). Peptidase enzymes also have been reported in the midgut of several aphid species (Srivastava, 1987), and leucine aminopeptidase has been found in *A. pisum* (Rahbé *et al.*, 1995) as well as being detected in the midgut of Aleyrodidae, *Aleurotrachelus socialis* (Bondar) and *Bemisia tabaci* (Gennadius) biotypes A and B (P.-A. Calatayud, unpublished data). The principal nitrogenous sources in the phloem sap are free amino acids (Ziegler, 1975), some of which are essential in the

development of phloem feeders. Since the phloem sap contains also oligopeptides (Auclair, 1963; Ziegler, 1975; Rahbé *et al.*, 1990), essential amino acids can be provided as a result of the action of digestive aminopeptidases, suggesting that such enzymes are important in the Sternorrhyncha. Additional research is required to confirm this hypothesis, however.

The oligophagy of *P. manihoti* and *P. herreni* towards cassava suggests that these insects are adapted to the secondary compounds of their host plant, particularly to linamarin, the characteristic secondary compound of cassava (Conn, 1980). This in turn suggests the presence of an enzymatic adaptation. Linamarase was found to be translocated by the phloem sap of cassava and ingested by the mealybug *P. manihoti*. Beta-glucosidase (linamarase, EC 3.2.1.21) activity was found in mealybugs reared on cassava plants (Calatayud, 1993; Calatayud *et al.*, 1994b; Calatayud *et al.*, 1997). Since no linamarase activity was found in *P. manihoti* reared on artificial diet containing linamarin (Calatayud, 2000), it can be surmised that the linamarase activity found previously in *P. manihoti* on plants was not biosynthesized by the insect itself.

Most aphids and mealybugs are known to possess symbiotic bacteria in bacteriocytes found in the haemocoele (Buchner, 1965; Tremblay, 1989). Apart from these haemocoele symbionts, some aphids have been reported to occasionally harbour gut microorganisms acquired from fortuitous plant surface contamination by the insect during the feeding process (Srivastava & Rouatt, 1963; Grenier *et al.*, 1994; Harada *et al.*, 1996). These microorganisms are generally absent in artificial diets. Being a phloem feeder, cassava mealybugs possess a specialized digestive system that allows excretion of large amounts of sugar sap in the honeydew, which provides a suitable medium for the growth of microorganisms living on the plant surface. Such a commensal relationship between mealybugs and microorganisms has been reported for the pink sugarcane mealybug, *Saccharicoccus sacchari* Cockerell (Sternorrhyncha: Pseudococcidae)(Inkerman *et al.*, 1986; Ashbolt & Inkerman, 1990). Because honeydew also contains linamarin (Calatayud *et al.*, 1994b), and linamarin is present on the cassava phyloplane (Renard, 1999), it is probable that the microorganisms associated with the cassava phylloplane possess an adapted enzyme system and therefore exhibit a linamarase activity.

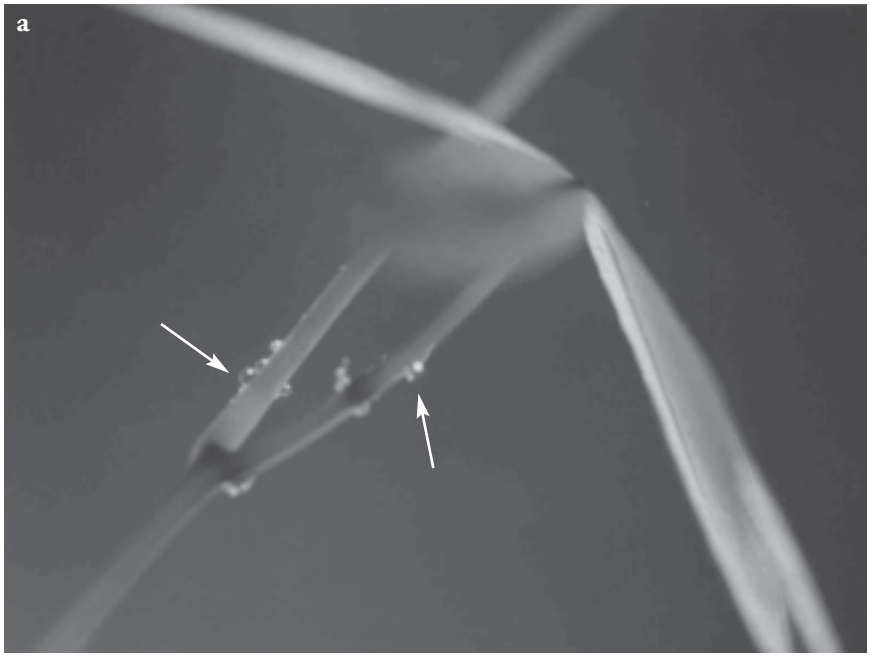
In *P. manihoti*, some bacteria (probably originating from the midgut) have been isolated from dissected insects; these are mostly Gram-negative bacilli related to the Enterobacteriaceae and Pseudomonadaceae (genus *Pseudomonas*) families

(Calatayud, 1993). The diameter of the alimentary canal of the stylets is estimated to be 2 µm (Figure 2.5), a sufficient width to allow bacteria from the cassava plant surface to pass through into the mealybug midgut. Moreover, all bacteria isolated from *P. manihoti* have been found to be cyano-tolerant. A tolerance of up to 750 ppm of cyanide in the medium in addition to possession of linamarase activities has been established in the bacteria occurring in the gut of *P. manihoti* (Calatayud, 1993), indicating that these bacteria are well adapted to cassava, are associated to this plant species surface, and likely originate from fortuitous contamination during the insect feeding process or are simply associated with the mealybug's body. Regardless of the bacterial origin, the microorganisms associated with mealybugs are mostly responsible for the linamarase activity reported previously in *P. manihoti* reared on fresh cassava plants. Yeast strains belonging to the *Rhodosporidium* genera and *Cryptococcus laurentii* were also isolated from the insects exhibiting linamarase activity and high adaptation to hydrogen cyanide (HCN) (P.-A. Calatayud, unpublished data). Moreover, no beta-glucosidase activity has been evidenced in the midgut of *P. herreni* (Figure 3.5). We can therefore conclude that no enzymatic adaptation towards linamarin occurs in cassava mealybugs.

NUTRITIONAL REQUIREMENTS

Biochemical constituents of the ingesta

The most viable technique in the collection of phloem sap has proved to be the stylectomy (Rahbé *et al.*, 1990). Used mostly with aphids, the technique involves cutting of the labium protecting the stylets during feeding and collection of the sap exuding from the amputated stylets (Downing & Unwin, 1977; Unwin, 1978; Kawabe *et al.*, 1980). The technique unfortunately cannot be used with mealybugs, as the insect's body is so close to the plant surface that it is not possible to see the labium. However, cassava is known to produce natural exudates (Figure 3.7a) which drop from the petioles, and the collection of such exudates is considered to be a good method of acquiring cassava phloem sap. These natural exudates are thought to be phloem secretions from companion cells, as no structural nectaries are detectable at the exudation sites (Pereira & Splittstoesser, 1987).



P.-A. Calatayud

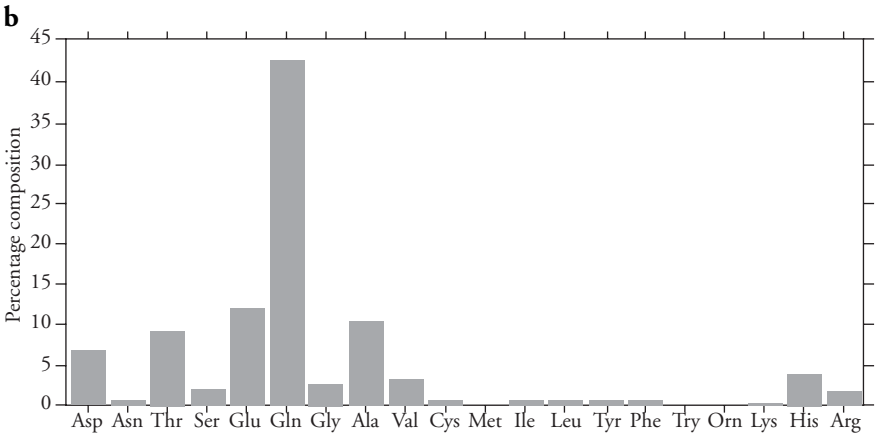


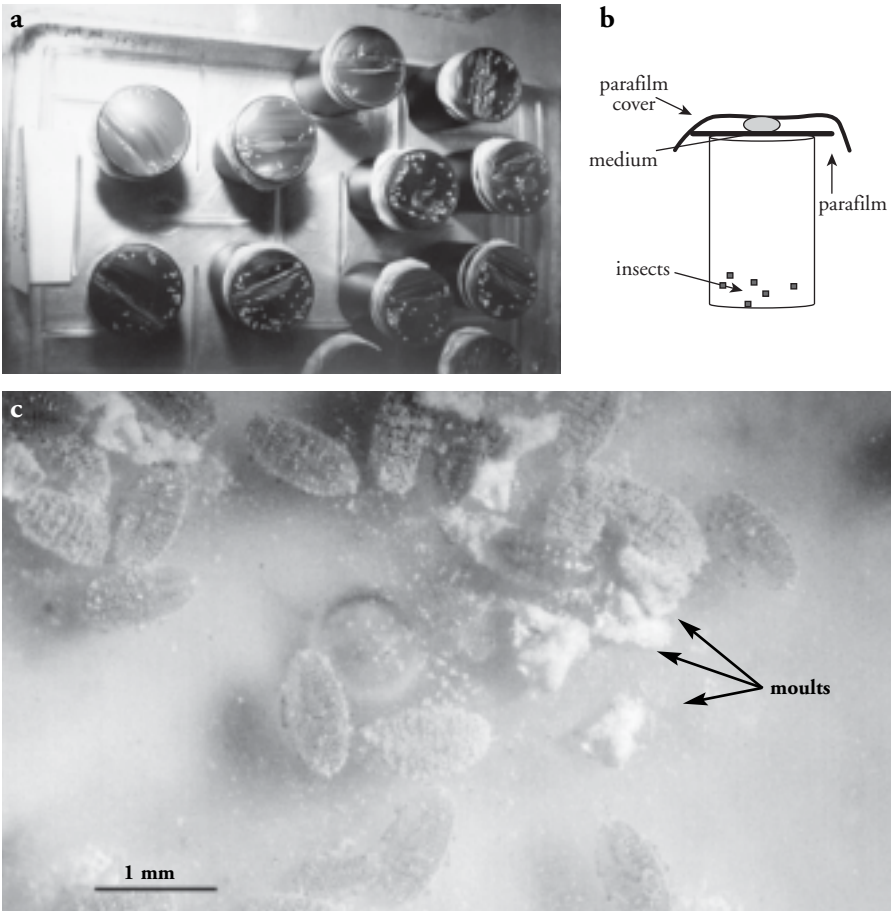
Figure 3.7
Natural exudates along the petiole of cassava (a) (see arrows) and the free amino acid composition of these exudates (b)

Analysis of the exudates shows that free sugars (sucrose, fructose and glucose) and free amino acids are the major compounds present (Pereira & Splittstoesser, 1987; Calatayud, 1993). As mentioned previously, the major constituent of cassava phloem sap is sucrose, accounting for 70% of the total compounds detected. Free amino acids, the principal nitrogenous compounds in phloem, are often characterized by an exceptionally unbalanced composition (Ziegler, 1975). Glutamine and/or asparagine are the main amino acids in the phloem sap of many plant species (Sasaki *et al.*, 1990; Girousse & Bournoville, 1994; Sandström & Petterson, 1994). In cassava, glutamine alone accounts for 42% of the total amino acids, followed by glutamic acid (~12%), alanine (~10%), threonine (~9%) and aspartic acid (~7%) (Figure 3.7b) (P.-A. Calatayud, unpublished data). Due to this disproportionate prevalence of certain free amino acids, the amino acid composition of the diet is likely a crucial factor in mealybug metabolism.

Artificial diets

A proper balance of amino acids is essential in maintaining the quality of an artificial diet for Sternorrhyncha, and aphids in particular. Studies based on carcass analysis of an aphid species *Acyrthosiphon pisum* Harris (Sternorrhyncha: Aphididae) have led to the development of a rearing medium that is adequate for the developmental requirements of many aphid species (Febvay *et al.,* 1988). This medium essentially contains free amino acids, sucrose, vitamins, cholesteryl benzoate and oligoelements, and has been developed and modified to suit the rearing of *P. manihoti* and *P. herreni* by Calatayud and colleagues (1998; 2002a). The diet is useful in testing for potentially active molecules and in identifying the phagostimulants which make the diet acceptable to the insects. The rearing technique involves the enclosure of the liquid diet in a sterile Parafilm sachet as previously described by Srivastava & Auclair (1971) (Figure 3.8).

Using this rearing technique, Calatayud and co-workers (2002a) demonstrated for the first time in a Pseudococcid species that among the 20 amino acids commonly required for protein synthesis, as well as ornithine, certain amino acids play a phagostimulatory and/or a nutritive role in *P. herreni*. Aspartic acid, glutamic acid, valine and alanine play a major phagostimulatory role, while lysine, ornithine, asparagine, methionine and histidine have a nutritive function in *P. herreni*. Other amino acids such as glutamine, cysteine, tryptophan, glycine and arginine have both phagostimulatory and nutritive functions.



P.-A. Calatayud

Figure 3.8
Rearing units used to rear *Phenacoccus manihoti* and *P. herreni* on a holidic liquid diet (according to Calatayud, 2000 and Calatayud *et al.*,1998; 2002a). The rearing unit (a) consists of an enclosed liquid medium sandwiched between two pieces of Parafilm to make a sachet (b), which is then mounted on the top of a black standard film cannister (height, 5 cm; diam, 3.2 cm). Larvae are then introduced into the open film tin, the tin capped with the Parafilm sachet and incubated under low light in a vertical position. The larvae feed through the horizontal membrane. In (c), *P. manihoti* larvae grow under artificial conditions

Amino acids are generally considered as essential if the insect's development ceases altogether or is markedly reduced when omitted from the diet. Calatayud and colleagues (2002a) observed that *P. herreni* development is consistently delayed when glutamine, cysteine, tryptophan, lysine, ornithine, asparagine, glycine, methionine or arginine are absent in the diet, proving that these nine amino acids are essential for development. For such insects, the consideration of whether or not an amino acid is essential appears to be complex, however, because this may not be due to an inability to biosynthesize them, but rather due to an overly slow conversion rate, as previously proposed by Febvay and coworkers (1995) for aphids. In fact, the amino acid composition of a carcass of a mealybug fed on a diet omitting a single amino acid was identical to that of an insect maintained on a control diet (*i.e.* containing all the amino acids)(Calatayud *et al.*, 2002a).

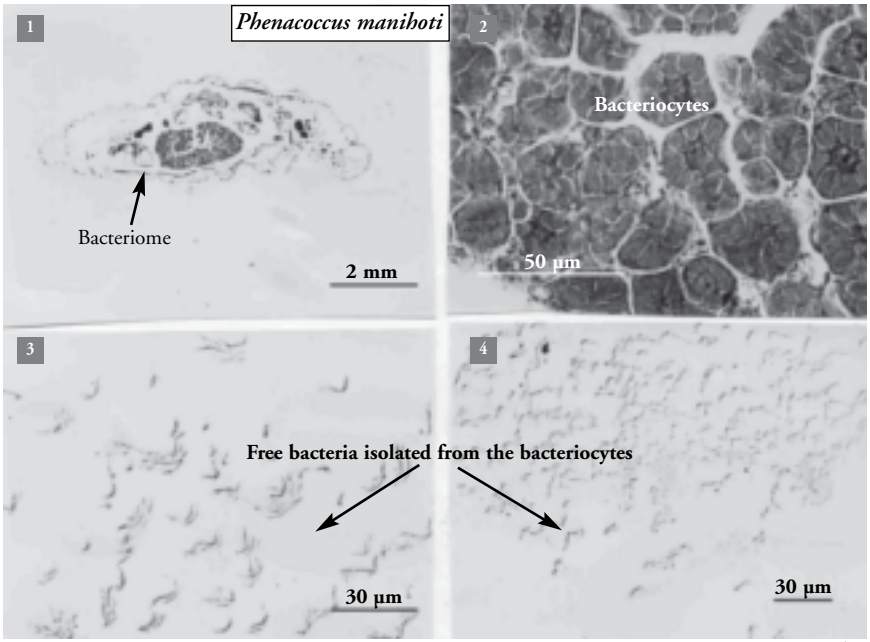
Similarly to other Sternorrhyncha, phagostimulants play a very important role in plant-mealybug interactions (see Srivastava, 1987 for review). Sucrose, generally considered to be a strong phagostimulant in Sternorrhyncha, has contributed significantly to the successful rearing of mealybugs on holidic diets (Gothilf & Beck, 1966; Calatayud *et al.*, 1998; 2002a). In aphids, certain amino acids, either alone or in combination, act synergistically with sucrose as phagostimulants (Srivastava, 1987). Such synergistic phenomena occur also in *P. herreni* (Calatayud *et al.*, 2002a). Other constituents of the artificial diet important for mealybug growth and development include vitamins, cholesteryl benzoate and oligoelements.

Symbionts

The identity of Pseudococcidea symbionts is not completely clear. Although bacterial origin is frequently mentioned, yeast origin is also reported (Buchner, 1965; Tremblay, 1989). The cassava mealybugs possess a bacteriome with bacteriocytes known to harbour symbionts (Figure 3.9a and b). As it has been difficult to obtain aposymbiotic *P. manihoti* mealybugs using rifampicin antibiotic (P.-A. Calatayud, unpublished observation), it is probable that *P. manihoti* possess not only bacteria as symbiotic microorganisms, but also yeast.

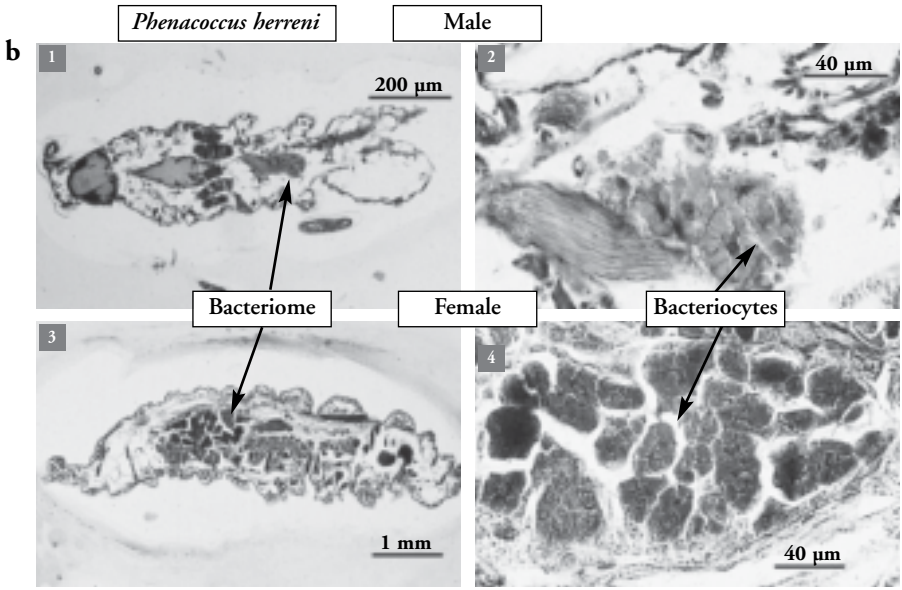
In *P. herreni*, a sexual species, the males also possess a bacteriome with bacteriocytes likely harbouring symbionts (Figure 3.9b). The adult male's bacteriome is smaller than that of the adult female, probably due to the absence of feeding and thus of digestive activity in the adult males (see Chapter 2 on feeding behaviour). In both *P. manihoti* and *P. herreni*, these symbionts most probably provide all the essential amino acids necessary for growth and development, and are evolutionarily associated with the unbalanced amino acid composition of the insects' diet.

a



C. Nardon

Figure 3.9a
Micrographs showing the bacteriome in *P. manihoti* (a). The longitudinal section of the *P. manihoti* body in (a-1) shows the bacteriome constituted by the bacteriocytes (a-2), from which the symbiotic bacteria can be isolated (a-3 & a-4).



C. Nardon

Figure 3.9b
Micrographs showing the bacteriome in *P. herreni* (b). A longitudinal section of the body of a *P. herreni* male (b-1) shows the bacteriome constituted by the bacteriocytes (b-2), while below, a longitudinal section of the body of a *P. herreni* female (b-3) shows the bacteriome with bacteriocytes (b-4)

4 Influence of Climate on Cassava–Mealybug Interactions

The main climatic constraint for cultivated crops in the tropics is the presence of long periods of drought. Insect densities often increase under such conditions of environmental stress (White, 1974; Mattson & Haack, 1987; Koricheva *et al*., 1998). Several reasons have been advanced to account for such increases, including improved food quality (White, 1974; 1984), reduced efficiency of natural enemies, and direct effects of the abiotic environment (Mattson & Haack, 1987). Of the above, food quality has received most attention (Mattson & Haack, 1987; Larsson, 1989; Koricheva *et al*., 1998). Biochemical changes leading to enhanced insect performance have been shown to take place in plants under abiotic stress, for instance increases in concentrations of soluble amino acids (White, 1974; 1984), thus possibly contributing to insect outbreaks (Mattson & Haack, 1987). This hypothesis has been criticized by Larsson (1989) and Koricheva *et al*. (1998), who suggest that stress from water limitation either has nil or a negative effect on insects. A possible explanation for these inconsistent findings is the fact that insects differ in their feeding modes and should therefore respond differently to changes in food quality under stress conditions (Mattson & Haack, 1987; Larsson, 1989). This is exemplified by the response of phloem-feeding insects, which is stronger (*i.e*. an enhanced performance) compared to that of chewing and leaf-feeding insects (Larsson, 1989; Larsson & Björkman, 1993; Koricheva *et al*., 1998).

In Africa and South America, outbreaks of natural populations of *P. manihoti* and *P. herreni,* respectively, occur on cassava every year during the dry season (Fabres, 1981; Bellotti *et al*., 1983; Noronha, 1990; Le Rü *et al*., 1991). For example, the number of *P. manihoti* individuals multiplies within 7 to 10 weeks from less than 10 individuals per plant (the number usually seen in the rainy season) to about 100 per plant. These dramatic changes in number occur together with important modifications in plant physiology (*e.g.* cessation of development of the plant) at the onset of the period of water stress (Le Rü *et al*., 1991). These observations suggest that some changes in the trophic quality of cassava positively influence the abundance of mealybugs, expressed as a temporary alteration in resistance against the insect during the dry season. Although it might be thought that the strong decrease of mealybug populations at the beginning of a rainy season is due to the mechanical action of the rain. However, using a rainfall

simulator, Le Rü and Iziquel (1990) demonstrated that although rain acts mechanically to produce variations in *P. manihoti* numbers, it is not a unique cause of the fast decrease in mealybug populations generally observed during the beginning of a rainy season. These authors conclude that the mechanisms responsible are probably numerous and complex, including not only the mechanical action of the rain, but also the impact of disease, the action of natural enemies, and the changes in host-plant physiology.

To test the hypothesis that the frequently observed increase in mealybug populations on cassava during periods of drought occurs as a result of cassava's undergoing important modifications in plant nutrients, the physiological modifications of cassava to resist water limitation stress were studied and their influence on mealybug growth and development investigated.

INFLUENCE OF DROUGHT STRESS ON CASSAVA

Cassava can endure several months of drought during its seasonal cycle. Although some cassava varieties are more tolerant to drought stress than others, *Manihot esculenta* is generally highly resistant to long dry seasons (El-Sharkawy, 1993). This is achieved by the plant's reducing its evaporative surface (El-Sharkawy, 1993). Calatayud and colleagues (2000a, b) have shown under laboratory conditions that the stems stop growing from the onset of the period of water limitation stress, while the number of leaves per plant decreases due to a dramatic acceleration of leaf senescence and leaf drop in addition to a substantial decrease in leaf emergence (Figure 4.1); the leaf area that emerges and expands during water limitation is about half of that in the control. All these modifications lead to a substantially reduced leaf canopy.

Similarly to other plants in general, cassava also resists drought by enhancing water use efficiency through stomatal closure (El-Sharkawy, 1993). Stomatal conductance is very low under water-limiting conditions, whatever the leaf age. A small transitory increase is nevertheless detectable in young leaves at the beginning of the morning, explaining the remaining maintenance photosynthetic rate under water stress conditions (Calatayud *et al.*, 2000a, b). The leaves of cultivated cassava as well as of wild species are predominantly hypostomatic, *i.e.* with stomata on the lower face of the leaves (P.-A. Calatayud, unpublished observation). A few varieties and species, how-

P.-A. Calatayud

Figure 4.1
Four-month-old cassava plants grown on a mixture of standard peat and sand under glasshouse conditions and optimal water availability (control) (1), or after 45–48 days of water limitation (stressed) (2) according to Calatayud *et al.* (2000a, b). Water stress was imposed by lowering the irrigation volume

ever, possess amphistomatic leaves with stomata on both the upper and lower surfaces, but with a low stomatal density on the upper face (Figure 4.2). This anatomical characteristic of having stomata mostly on the lower leaf face may contribute to a favourable adaptation of cassava to water stress by limiting evaporation.

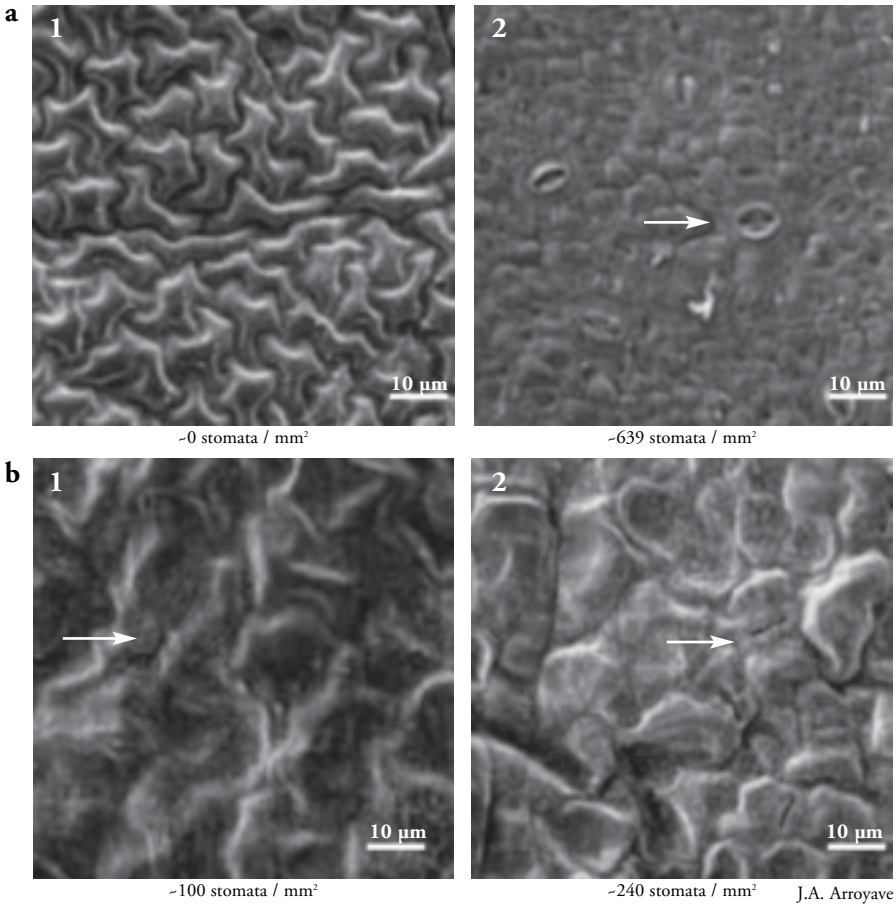


Figure 4.2
Leaf surfaces of a hypostomatic species, *Manihot crassisepala* (a) and an amphistomatic species, *Manihot rubricaulis* (b) showing stomata (see arrows) dispersed on the upper (1) and/or the lower (2) leaf surface

Another physiological response of cassava to long periods of drought is an increase in cell metabolite concentrations, which probably works to maintain the osmotic pressure of the tissues for protection of cellular structures (Calatayud *et al.*, 2000a). The total amount of glucose, fructose and sucrose in leaf tissues increases following water limitation. In addition, the free amino acid level increases, with serine, glutamic acid, glutamine and arginine concentrations reaching very high levels. An increase in the levels of some organic acids such as malate, succinate and citrate was also observed. The increase in the levels of sucrose and organic acids in leaves of water-depressed plants is associated with higher activities of sucrose phosphate synthase and phosphoenolpyruvate carboxylase (PEP case), respectively.

All of these mechanisms of stress avoidance (reduction of the evaporative surface, efficient stomatal closure and increase in cell metabolite concentration to maintain cell osmotic pressure) are the main mechanisms commonly used by plants. However, they are extremely effective in cassava, explaining in part why this plant is highly resistant to long periods of drought.

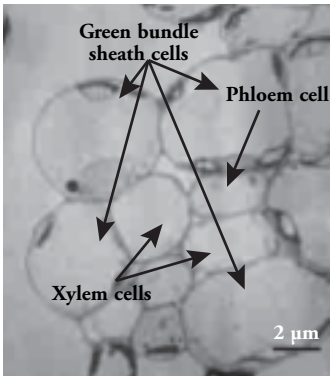
PHOTOSYNTHESIS IN CASSAVA

Atmospheric carbon dioxide (CO_2) is generally assimilated into 3- or 4-carbon compounds by plants, giving rise to type C_3 or C_4 photosynthetic plants. Since in C_4 photosynthetic plants water-use efficiency is increased and photorespiration is suppressed, C_4 plants are often more competitive than C_3 plants in drought areas and in warm environments (Edwards & Ku, 1987). In cultivated cassava, the photosynthetic CO_2 assimilation pathway has been a matter of debate (Angelov *et al.*, 1993). Early work classified cassava as a C_3-C_4 intermediate (El-Sharkawy & Cock, 1987). Later, it was demonstrated that the plant displayed C_3 photosynthesis (Edwards *et al.*, 1990). Nevertheless, leaves of *Manihot* species have distinct green bundle sheath cells (cells with chloroplasts immediately surrounding the phloem cells, a typical anatomical feature of C_4 plants) which are unusual in type C_3 leaves (Angelov *et al.*, 1993) (Figure 4.3).

The difficulty in determining the photosynthetic CO_2 assimilation pathway in cassava could be due to the fact that *M. esculenta* is thought to be derived from wild species possessing C_4 photosynthesis. Studies by Calatayud and co-workers (2002b), however, have demonstrated that most of the *Manihot* species, including *M. flabelli-*

folia—considered to be the wild progenitor of cultivated cassava, *M. esculenta* (Fregene *et al.*, 1994; Roa *et al.*, 1997)—exhibit typical C3 characteristics. This is evidence that cultivated cassava is not derived from wild species possessing C4 photosynthesis.

In conclusion, cassava does not possess a particular photosynthetic metabolism that enables the plant to resist drought, but it does exhibit efficient mechanisms limiting evapo-transpiration and water loss, as well as maintaining the osmotic pressure of the leaf tissues.



J.A. Arroyave

Figure 4.3
Distinct green bundle sheath cells in leaves of *Manihot rubricaulis*, observed by transmission electronic microscopy

INFLUENCE OF WATER LIMITATION ON PLANT–MEALYBUG INTERACTIONS

Under controlled environmental conditions, *P. manihoti* exhibits the highest intrinsic rate of increase of plants under drought stress, indicating that cassava grown under water shortage enhances *P. manihoti* performance (Fabres & Le Rü, 1988). Water limitation induces changes in cassava physiology that also favour *P. herreni* development and reproduction (Calatayud *et al.*, 2002c). The fecundity and adult weight of mealybug females are higher on plants under water stress. The insects also grow faster and the maturation of females (evidenced by the start of egg-laying into an ovisac) is faster on plants under water limitation stress, as illustrated in Figure 4.4.

The faster development of *P. herreni* on water-stressed plants can be related to nutritional factors. An increase in the level of sucrose and free amino acids in the artificial diet favours mealybug growth (P.-A. Calatayud, unpublished data). Moreover, some amino acids such as glutamine and arginine, whose levels increase strongly in cassava leaves under water limitation, play an important role in the development of the *P. herreni* female; for example, when these constituents are absent in the diet, the insect does not grow to the adult stage (Calatayud *et al.*, 2002c). This demonstrates

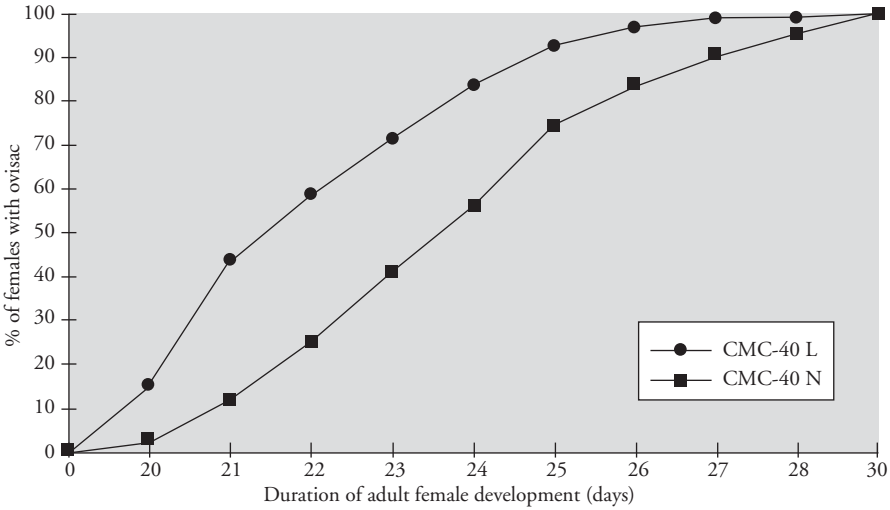


Figure 4.4
Accumulated percentage of mature female (*i.e.* beginning to form ovisac) of *Phenacoccus herreni* reared on cassava (var. CMC 40) grown with normal (N) or low (L) water availability

the importance to mealybug growth of increased levels of free amino acids such as glutamine and arginine in the mesophyll cells, and consequently in the phloem sap, of cassava leaves under water limitation.

Table 4.1
Weight (means[1] ± SE) of *Phenacoccus herreni* females reared on different diets after 35 days

Dietary ratio of sucrose/ free amino acids[2]	Weight (mg)	Number of females observed
Ratio 5	0.244 ± 0.009 a	110
Ratio 2.5	0.384 ± 0.014 b	76

[1] Means followed by different letters are different at the 5 % level (Mann-Whitney U test).
[2] Molar ratio (5, found on well-irrigated cassava; 2.5, found on drought-stressed plants).

The molar ratio of sucrose to free amino acids in cassava leaves has been shown to decrease by half under conditions of low water availability (Calatayud *et al.*, 2000a). Studies using artificial diets with two distinct sucrose/total free amino acids ratios (5 and 2.5), showed that the 2.5 diet ratio (corresponding to the molar ratio found in cassava leaves under low water availability) favours mealybug growth (Table 4.1) (CIAT, 1999). The weight of adult females was significantly higher on this diet, and this is due to a more balanced ratio of sucrose to free amino acids.

5 Host-Plant Resistance

Plants have developed various mechanisms that reduce their likelihood of being consumed or damaged by herbivores. Plant defence mechanisms can be categorized into direct defence, which include antixenosis and antibiosis (also called intrinsic mechanisms), and indirect defence (extrinsic mechanisms) (Price *et al.,* 1980). Direct defensive mechanisms include morphological features (e.g. trichomes or hairs, thorns, waxes) and chemical adaptations (production of toxins, repellents, digestibility reducers) that have a direct negative impact on herbivore attack and survival. Earlier investigations and assessments have resulted in the incorporation of this knowledge into agricultural production practices for years, as demonstrated by the use of resistant crop varieties.

Indirect defence mechanisms are related to those traits promoting the effectiveness of the natural enemies of herbivores; they involve the provision of alternative food (nectar), shelter (domatia) or information on herbivore presence and identity (semiochemicals). The efficacy of these natural enemies is sometimes so spectacular that they are referred to as the plant's "bodyguards" (Dicke & Sabelis, 1988). Indirect defence mechanisms have been well documented over the past 20 years and this has led to the development of biological control practices in many crops. It is now accepted that there is a strong link between intrinsic and extrinsic defense mechanisms, thereby underscoring the importance of managing plant attributes from a tritrophic perspective (Thomas & Waage, 1996; Cortesero *et al.,* 2000).

INTRINSIC MECHANISMS

Painter (1951) categorized the intrinsic mechanisms of plant defense into three, namely - antixenosis (or non-preference), which arises from the plant to deter or reduce initial colonization by insects. This mechanism can be due to biophysical or biochemical factors or a combination of both;

- antibiosis, which operates after the insects have colonized and have started utilizing the plant. This mechanism of resistance generally affects the insect's growth, development, reproduction and survival. Chemicals and/or physical factors can be involved in antibiosis;
- tolerance, which refers to the resultant effects and not the mechanisms. This is a genetic trait of adaptation of a plant to insect infestation which prevents economic yield loss or lowering of the quality of the plant's marketable product.

With reference to cultivated cassava, *Manihot esculenta*, no total resistance resulting in a complete absence of mealybugs has been identified (Le Rü & Calatayud, 1994; Bellotti *et al.*, 1999). Varietal differences in partial resistance to *P. manihoti*, including antixenosis, antibiosis and tolerance, have been observed, however (Le Rü & Tertuliano, 1993; Tertuliano *et al.*, 1993). These results suggest that there are many resistance mechanisms operating in cultivated cassava, and that cassava resistance is of the horizontal type and is polygenic, as previously proposed for resistance to *P. herreni* by Bellotti and Kawano (1980).

It is well documented that secondary compounds in addition to nutritional factors can play important roles in the resistance of plants to insect pests (Fraenkel, 1969; Kogan, 1977; Pickett *et al.*, 1992). In Sternorrhyncha, numerous studies on aphids have illustrated the involvement of alkaloids (Smith, 1966; Dreyer *et al.*, 1985; Wink & Witte, 1991), phenolic acids and flavonoids (Todd *et al.*, 1971; Dreyer & Jones, 1981; Mc Foy & Dabrowski, 1984; Leszczynski *et al.*, 1985) or cyanide compounds (Schoonhoven & Derksen-Koppers, 1976; Dreyer & Jones, 1981) in plant resistance. The role of secondary compounds (metabolites) is generally highly dependent on their localization within the plant tissues. Plant compounds strictly located in the mesophyll are thought to have a greater chance of playing a deterrent role during stylet penetration, an example of antixenotic resistance. On the other hand, if located in the phloem, these compounds will influence settling or feeding depending on their behavioural or metabolic effectiveness —a case of either antixenotic or antibiotic resistance (Givovich *et al.*, 1992). As reported in Chapter 2 on host-plant selection, the secondary compounds found in *M. esculenta* are cyanogenic glucosides (mostly linamarin) as well as phenolic acids and flavonoids (Conn, 1980; Calatayud *et al.*, 1994b). No alkaloid has been detected.

Antixenosis

Linamarin, present on cassava phylloplane, is involved in initial host recognition by *P. manihoti* (see Chapter 2 for details). This compound is also present in the phloem

sap, and plays a phagostimulation role to the insect (Calatayud *et al.*, 1994a; Renard, 1999; Calatayud, 2000). These authors reported also that cassava varieties with higher levels of linamarin are generally more preferred by the mealybugs. In contrast, less preferred cassava genotypes have higher levels of phenolic acids in their cell wall structures which most likely inhibit the salivary enzyme activities during the penetration process of the stylets. This explains in part the delay in reaching the phloem by *P. manihoti* stylets on such cassava genotypes (Calatayud *et al.*, 1994a).

Antixenosis is generally regulated by a balance between attractant and repellant compounds from the plants (Schoonhoven *et al.*, 1998). In cassava, the balance between linamarin and phenolic acid levels in the leaves seems to be a good indicator in predicting mealybug preference for the plant variety.

Antibiosis

The phenomenon of cyanogenesis which occurs in cassava was formerly assumed to provide protection to the plant against phytophagous insects (Hruska, 1988). Following mechanical damage to the tissues by insects, hydrocyanic acid (HCN) is released as a result of linamarin coming into contact with linamarase (Conn, 1980). The location of linamarase in cassava leaves is a matter of debate. Mkpong and colleagues (1990) showed that this enzyme has an apoplastic location, but later Pancoro and Hughes (1992) found the enzyme to be located principally in laticifers (latex vessels). Whatever the location of this enzyme, the substrate (linamarin) is present in other tissue locations, indicating that the substrate and enzyme are compartmentalized and come into contact after tissue damage.

Cyanogenesis in cassava is not a mechanism that protects the plant against mealybugs, however, because it is probably not induced by insect infestation. The penetration process of the insect's stylets induces little cellular damage (Calatayud *et al.*, 1994a; 2001a); it is therefore unlikely that linamarase can come into contact with linamarin for cyanogenesis to occur after stylet penetration. Studies on *P. manihoti* report that the linamarin ingested is probably hydrolysed by the bacteria occurring freely in the midgut, which would explain the appearance of free cyanide found in the honeydew of mealybugs (Calatayud *et al.*, 1994b). Free cyanide therefore does not come from cyanogenesis. Moreover, free cyanide has been shown to be not very toxic to this insect, due to its efficient excretion system (Calatayud *et al.*, 1994b) and/or to an efficient detoxification mechanism by the gut bacteria. No relationship has been established between the levels of primary compounds (amino acids and sug-

ars) of leaves and antibiosis to *P. manihoti* (Tertuliano & Le Rü, 1992), suggesting that other biochemical factors may be involved in antibiosis resistance of cassava.

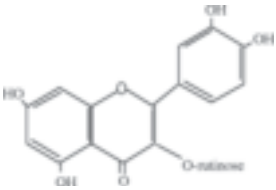


Figure 5.1
Rutin structure (rutinose = rhamnose + glucose)

Rutin (a flavonoid glycoside, Figure 5.1) has been shown to be translocated by the phloem sap of cassava, and is therefore consumed by *P. manihoti* (Calatayud 1993; Calatayud *et al.*, 1994b). No linear dose effect was found between rutin content and the antibiosis level in a study by Calatayud and coworkers (1994b). Poinsettia (*Euphorbia pulcherrina* Wild., Euphorbiaceae) and talinum (*Talinum triangularae* Jacq., Portulacaceae) are optional hosts of *P. manihoti* (Calatayud *et al.*, 1994a), the latter being infested only if high infestations on adjacent cassava plants occur (F. Schulthess, ICIPE, personal communication). The two plants display marked differences in antibiosis when tested in the laboratory (Tertuliano *et al.*, 1993). The rutin content in the leaves is 13-fold higher in 'resistant' poinsettia (rutin at 16 mg/g; r_c[1] = 0.038) than in 'susceptible' talinum (rutin at 1.2 mg/g; r_c = 0.150)(Calatayud *et al.*, 1994b). In addition, rutin levels are sensitive to the presence of the mealybug, regardless of the plant genotype (Calatayud *et al.*, 1994b). This observation has been confirmed by independent experiments involving increased levels of infestation (P.-A. Calatayud, unpublished data).

The biological activity of rutin towards *P. manihoti* has been studied using artificial diets (Calatayud, 2000). Introducing commercial rutin at various concentrations into a liquid diet has shown that the presence of this compound in the diet affects mealybug growth and development. The authors of this book, however, suggest that rutin seems to play an anti-nutritive function in the insect through phagodeterrency, thus delaying development to the adult, rather than having a toxic effect (P.-A. Calatayud and B. Le Rü, unpublished data). The appearance of quercetin (the rutin aglycone) in the honeydew of *P. manihoti* (Calatayud *et al.*, 1994b) indicates that deglycosylation of rutin probably occurs in the insect and suggests that this hydrolysis product (quercetin) should be more toxic to the insect's physiology than the quercetin glycoside, *i.e.* rutin itself. Metabolism of phenolic compounds has also

[1] rc: intrinsic rate of increase of insect populations (after Laughlin, 1965). The value of this mathematical parameter is lowest on plants exhibiting the highest antibiosis.

been proposed to occur in the aphid *Macrosiphum rosae* (L.)(Peng & Miles, 1991). It is probable that the mealybug's intestinal flora is involved in the hydrolysis of rutin into quercetin by the action of rutin glycosidase, as reported for the bacterium *Pseudomonas viridiflava* (Hendson *et al.*, 1992). Therefore, the fortuitous gut bacteria ingested by the insect play a negative role in mealybug–cassava interactions, being more involved in the plant's defence mechanism rather than protecting their insect host. Additional research is needed to confirm this hypothesis.

The above studies all indicate the probable participation of rutin in antibiosis resistance of cassava to *P. manihoti*, or at least its linkage to an induced (defensive?) reaction of cassava towards the insect. This defensive reaction is manifested by an increase in the total phenolic compounds (mostly flavonoids, including rutin) after mealybug infestation (Calatayud *et al.*, 1992; Calatayud *et al.*, 1994b; 1994c; Tertuliano *et al.*, 1999). Such a defensive reaction of cassava is also induced after infection by cassava bacterial blight (*Xanthomonas campestris* pv. *manihoti*), and is more intense in cassava resistant to the bacterium than in susceptible varieties (Kpémoua *et al.*, 1996; P.-A. Calatayud, unpublished data). An increase in rutin level in leaves appears to be a good indicator or prediction as to whether a cassava variety will be resistant or susceptible to insect infestation and/or bacterial infection.

The involvement of flavonoids in the resistance of plants to phloem-feeders has been extensively documented for aphids (Todd *et al.*, 1971; Dreyer & Jones, 1981; McFoy & Dabrowski, 1984). Rutin is an allelochemical that affects the growth, development, reproduction and metabolism of insect species other than *P. manihoti* (Beck & Reese, 1976). With reference to *Schizaphis graminum* (Sternorrhyncha: Aphididae), Dreyer and Jones (1981) suggest that in phloem-feeding insects, secondary compounds probably not only affect the insects themselves but also their symbionts. Similarly, the physiology of the endosymbiotic bacteria harboured by *P. manihoti* (see Chapter 3) should likely be disturbed by ingested and metabolised rutin. Additional data are needed to confirm this hypothesis.

Tolerance

Cock (1979) reported decades ago that cassava is relatively tolerant to pest attack. He suggested that tolerance is due to the extensive production of leaves during the growing season that compensates for the reduction in leaf production caused by the pest during the dry season, when the leaf area is already reduced due to drought. The

tolerance of cassava to *P. manihoti* has been measured by quantifying morphological and physiological changes in plant growth after infestation (Le Rü & Tertuliano, 1993). All the cassava varieties studied were generally tolerant to mealybug infestation, but with different degrees of tolerance. Some varieties exhibited both a relatively high tolerance and a strong antibiotic resistance, suggesting that such varieties could be selected for a breeding strategy for mealybug resistance.

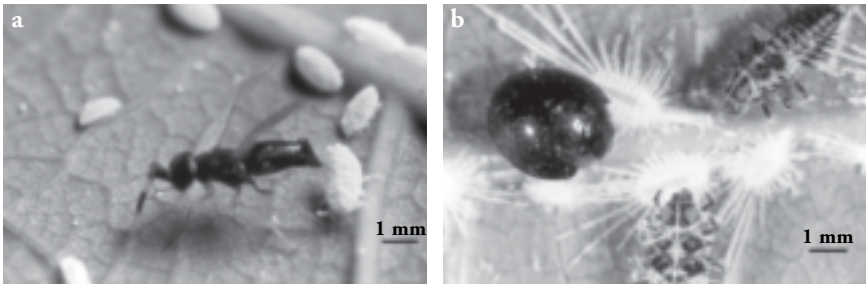
Total resistance

Although no total resistance to mealybugs has been evidenced in cultivated cassava, such resistance should be detected in wild cassava species or closely related species (Calatayud & Mùnera, 2002). In this context, 26 wild cassava species in addition to *Jatropha gossypiifolia* L. (Euphorbiaceae) were artificially infested with *P. herreni* under laboratory conditions. Only *J. gossypiifolia* exhibited a strong toxicity to the insect: after 48 hours of infestation, this plant showed 100% insect mortality. It was observed that mortality is not only due to the presence of glandular trichomes on the leaf surface, but also due to a toxicity most likely residing in the mesophyll cells. Moreover, this plant species appears to be highly phagodeterrent to *P. herreni*, as the larvae remain extremely mobile after infestation of the leaves (P.-A. Calatayud, unpublished observations). Therefore, *J. gossypiifolia* displays a total resistance to *P. herreni* by using both antixenosis and antibiosis mechanisms, resulting in a total mortality of mealybugs 48 hours after infestation. The resistance of *J. gossypiifolia* to *P. manihoti* has not been studied.

Jatropha gossypiifolia was initially confused with a wild species of cassava, and in fact it is genetically close to the *Manihot* genus (M. Fregene, CIAT, personal communication). The plant reproduces well by seeds, possibly making it useful in a breeding programme to obtain hybrids (with cassava varieties) totally resistant to the cassava mealybug, *P. herreni*.

Influence of antixenosis and antibiosis on natural enemies

Natural enemies of phytophagous insects function and develop in a multitrophic context (Price *et al.*, 1980; van Emden, 1991). Souissi and Le Rü (1997) studied the relationship between the cassava plant and the parasitoid *Apoanagyrus (Epidinocarsis) lopezi* De Santis (Hymenoptera: Encyrtidae) (Figure 5.2a) used in the biological con-



J.P. Nenon B. Le Rü

Figure 5.2
***Apoanagyrus lopezi* female stinging a *Phenacoccus manihoti* 4th instar (a). *Exochomus flaviventris* female and its 5th instar larvae feeding on *Rastrococcus invadens* Williams (Pseudococcidae)(b).**

trol of *P. manihoti* in Africa. They showed that cassava with a high level of antibiosis resistance has a deleterious effect on *A. lopezi* survival and development. These authors later observed that the size of this parasitoid species negatively correlates with plant resistance (Souissi & Le Rü, 1998). *Apoanagyrus lopezi* appears to perform better if cassava cultivars are selected for their strong antixenosis (non-preference) but low antibiosis characteristics.

Other studies have revealed that most life-history parameters of the generalist ladybird predator *Exochomus flaviventris* Mader (Coleoptera: Coccinellidae) (Figure 5.2b), the most active predator of *P. manihoti* in Central Africa (Fabres & Kiyindou, 1985; Iziquel & Le Rü, 1989), are greatly influenced by the host plant, showing that mealybugs feeding on higher-antibiosis cassava are less suitable prey for the coccinellid (Le Rü & Mitsipa, 2000). However, the voracity of *E. flaviventris* in this study was high on the cassava variety with the highest antibiosis resistance. This suggests that such a cassava variety may synergistically interact with the coccinellids to provide a significant level of mealybug control. The conclusions drawn are that antibiosis in cassava significantly affects the life-history parameters of the coccinellids without modifying their fitness, and thus provides better cassava mealybug control (Le Rü & Mitsipa, 2002).

Influence of abiotic factors on direct plant defences

While drought stress positively affects the biological performance of the mealybugs *via* the superior nutrition provided (see Chapter 4), water limitation negatively influences

the cassava's resistance to the insect. During the long dry season when the *P. manihoti* population increases and spreads, the linamarin content in the leaves increases, producing cassava plants that are phagostimulant to the mealybug. Simultaneaously, the defensive response of cassava to *P. manihoti* infestation is less important during the dry season, explaining in part the changes in *P. manihoti* populations during this period (Calatayud *et al.*, 1994c) and suggesting the occurrence of a temporary alteration in partial plant resistance against the insect.

Infestation by *P. manihoti* is rarely observed in forest regions and in soils rich in organic elements (Matile-Ferrero, 1978; Nwanze *et al.*, 1979; Neuenschwander *et al.*, 1989; 1990). The impact of improved soil fertility in diminishing cassava infestation by *P. manihoti* has been reported also by Schulthess and colleagues (1997), and this finding has been supported by the evidence of Tertuliano and colleagues (1999) that mulch and manure are the best fertilizers in enhancing cassava resistance to *P. manihoti,* as shown by a higher defensive response (*i.e.* the increase of rutin level in leaves after infestation).

EXTRINSIC MECHANISMS

Plant and host-derived volatiles play a fundamental role both in herbivore habitat location and herbivore location by carnivores. The informational value of stimuli used by carnivores in the location of herbivores depends on two inversely correlated characteristics: detectability (*i.e.* degree of stimuli reception) and reliability in indicating herbivore presence and suitability. Volatile chemicals released in relatively large amounts from the food plant of the host provide long-range olfactory information to the carnivores; these compounds are easily detectable and mainly used in host habitat location. Volatile chemicals originating from the host itself and from its by-products and released in small amounts, appear to operate at short range and are involved in host location once the carnivore reaches the microhabitat of the herbivore (Vet & Dicke, 1992).

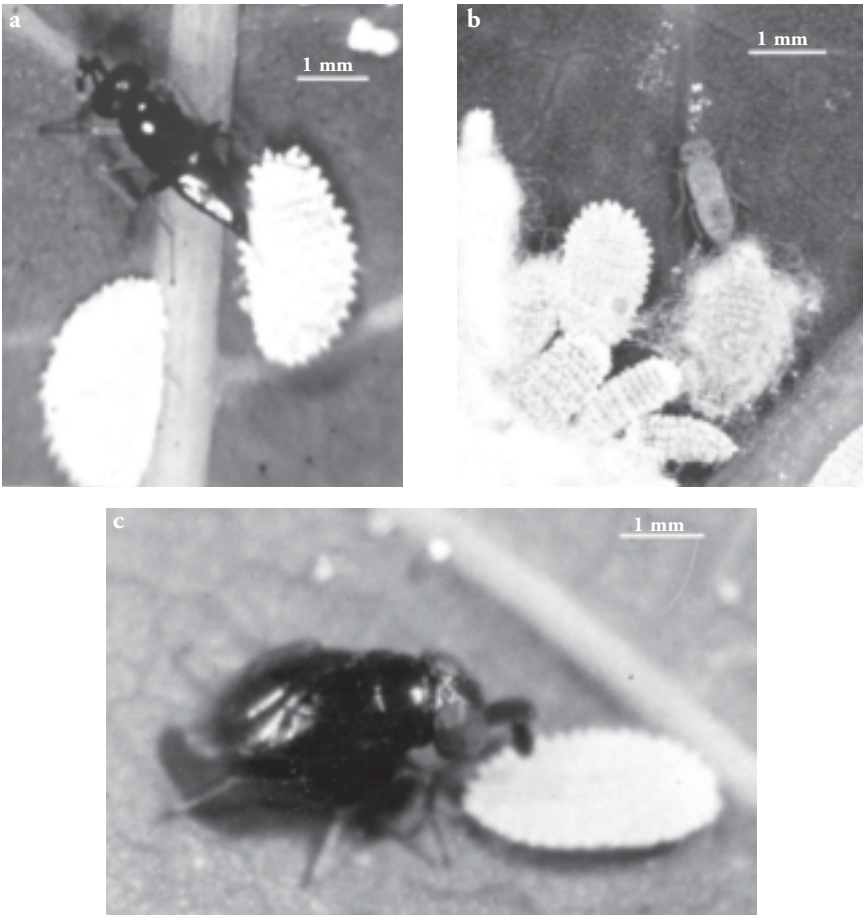
Some authors consider the release of chemical compounds that stimulate the attraction of the herbivore's natural enemies as an indirect mechanism of plant defence (Price *et al.*, 1980; Dicke *et al.,* 1990; Takabayashi *et al.*, 1995). Others propose this process to be a kind of antixenosis resistance of the plant against herbivores (Storer & van Emden, 1995; Petterson *et al.*, 1996). This indirect defence mechanism or

antixenosis resistance can be constitutive (Price *et al.*, 1980) or can be induced by herbivory (Dicke *et al.*, 1990; Takabayashi *et al.*, 1994). It is well documented that many natural enemies discriminate between volatiles produced by uninfested and infested plants (Turlings *et al.*, 1990; Reed *et al.*, 1995), by different plant species (Elzen *et al.*, 1986; Dicke *et al.*, 1990; Turlings *et al.*, 1990; Gouinguené *et al.,* 2001), and by different cultivars of the same plant species infested by the same herbivore (Elzen *et al.*, 1986; Dicke *et al.*, 1990; Takabayashi *et al.*, 1991, 1994; Gouinguené *et al.,* 2001). These complex behavioural responses indicate that olfactory information may be specific for different herbivore–plant interactions, suggesting that the quality and quantity of induced infochemicals may vary with the plant species or variety as well as with the herbivore. According to Takabayashi and colleagues (1991), the variation with the plant appears to be more important than with the herbivore.

Extrinsic mechanisms involving parasitoids

The responses of female parasitoids to volatile chemicals emitted by cassava infested by mealybugs have been investigated with the cassava – *P. manihoti* – *Apoanagyrus lopezi* model (Nadel & van Alphen, 1987; van Baaren & Nénon, 1996; Souissi & Le Rü, 1997), and with the cassava – *P. herreni* – *Apoanagyrus diversicornis* Howard / *Aenasius vexans* Kerrich / *Acerophagus coccois* Smith (Hymenoptera: Encyrtidae) model; the last three are the endoparasitoid species used to control *P. herreni* in South America (Bertschy *et al.*, 1997; 2001) (Figure 5.3). All these authors have clearly demonstrated that mealybug-infested cassava plants chemically mediate attraction of the parasitoid species.

Souissi and co-workers (1998) have shown that cassava plants damaged by *P. manihoti* are the main source of volatiles that attract females of *A. lopezi* to the microhabitat of its host. There is evidence that unparasitized mealybugs are more attractive than parasitized mealybugs, thus preventing the risk of superparasitism. Infested plants with unparasitized mealybugs attract more female parasitoids than plants infested with parasitized mealybugs. The data of these authors suggests that the source of attractants for female parasitoids at long distance originates from the interactions between the plant and feeding by the mealybug. These attractants are detected at longer distances than those produced by undamaged plants or kairomones – chemicals that convey information between two species and for which the receiver alone benefits from the exchange of information – left by unparasitized hosts, thus guiding the parasitoids into areas that harbour suitable hosts.



G. Guzman

Figure 5.3
***Apoanagyrus diversicornis* (a), *Acerophagus coccois* (b) and *Aenasius vexans* (c) females stinging *Phenacoccus herreni* 4th instars**

Bertschy and colleagues (1997) showed that *A. coccois,* a more generalist feeder than *A. diversicornis* and *A. vexans*, does not distinguish between healthy or infested leaves, suggesting that *A. coccois* may respond to general plant odour, whereas specialized parasitoids (*A. diversicornis* and *A. vexans*) respond more specifically to odours associated

with mealybug-infested plants. After landing on an infested plant, the mealybug-derived chemicals can play a role in host location. Calatayud and co-workers (2001b) have shown that *A. coccois* and *A. vexans* use a contact kairomone identified as O-caffeoylserine from the body surface of *P. herreni* as a host-location stimulant.

Extrinsic mechanisms involving predators

The importance of chemical signals emitted by damaged plants to the ability of natural enemies to detect hosts or prey has been extensively studied with reference to insect parasitoids and predatory mites (Vet & Dicke, 1992, Dicke & Vet, 1999; Cortesero *et al.,* 2000). However, it has been poorly documented in insect predators such as the coccinellids, although there is some evidence of their use in the case of the latter (Obata, 1986; Zhu *et al.,* 1999). The response of experienced gravid females of the generalist ladybird *E. flaviventris* to odours from the cassava–mealybug (*P. manihoti*) complex has been investigated by Le Rü and Makaya Makosso (2001). The odour of cassava plants alone was not attractive, but in dual-choice tests, the mealybug-infested plants were preferred to mealybugs alone and mealybug-damaged plants, and were the major sources of volatiles that attracted the female coccinellids to the microhabitat of their prey.

The authors emphasize that the emission of volatiles does not appear to be limited to the infested parts of the plant, but occurs systematically throughout the plant. The release of such induced compounds might be elicited by a factor present in the mealybug's saliva or by the feeding process of the insect. It has been revealed also that *E. flaviventris* females use herbivore-induced plant volatiles during foraging, and detect *via* olfaction the presence of conspecific gravid females and parasitized prey, thus assessing patch suitability from a distance. The presence of conspecific coccinellid larvae or adult males did not modify the attractiveness of the mealybug-infested plants, but the *E. flaviventris* females showed more preference for mealybug-infested plants with or without conspecific males than for mealybug-infested plants with conspecific predator females (alone or with conspecific males). Moreover, the female predators preferred the plant infested with unparasitized mealybugs over plants infested with parasitized mealybugs. These results confirm that ladybirds, which are very active predators with a full set of sensory organs, are capable of detecting small patches of mealybugs even when the pest is scarce in the habitat.

The involvement of plants in herbivore finding by the generalist coccinellid shows remarkable similarities with those of the specialized parasitoids. There are great

differences in the foraging behaviour of the parasitoid and predator, however: the specialized parasitoid *A. lopezi* shows an innate response to the systemically released cassava volatiles induced by mealybug infestation, while the generalist predator needs first to learn about them (J. P. Makaya Makosso, unpublished observation). This behaviour provides the parasitoid with a great advantage over the predator, as it can locate infested cassava immediately after emergence, whereas the female coccinellid needs at least 8 days before it can recognize cassava infestation.

Influence of abiotic factors on indirect plant defences

Indirect plant defences are thought to be modulated by environmental factors, however the mechanism of this is poorly understood (Cortesero *et al.*, 2000). Recently Koumba (2000) investigated the role of water stress and soil nitrogen on cassava's ability to attract both parasitoid (*A. lopezi*) and coccinellid (*E. flaviventris*). For both natural enemies, infested cassava under water limitation was preferred over infested cassava with a good supply of water, and both *P. manihoti* natural enemies preferred the odour of infested cassava supplied with nitrogen over that of infested cassava alone. Previous studies on tritrophic interactions have reported an increase of natural enemy densities on plants supplied with nitrogen (Hagen *et al.*, 1971; Fox *et al.*, 1990). These results suggest that both water stress and a good nitrogen supply increase the amount of synomone—chemicals that convey information between two species and for which both the receiver and the signaler benefit from the exchange of information—released by the infested plant and confirm that abiotic factors have a strong influence on the plant's ability to attract natural enemies. However, water stress appeared to enhance the encapsulation—immune response of mealybugs to parasitoids eggs or larvae—and to effect the success of parasitism and, depending on the parasitoid species, the parasitoid development as well. This has been observed for *P. herreni* and its associated parasitoids (Calatayud *et al.*, 2002c).

These studies also demonstrate that plants with good nitrogen supply have a beneficial impact on natural enemies. For example, soil fertility exhibits strong tritrophic-level effects *via* changes in the size of cassava mealybugs and the sex ratio of the parasitoid *A. lopezi* (Schulthess *et al.*, 1997). This corroborates the results of van Dijken and colleagues (1989) on sex allocation according to size of the mealybug. A strategy put forward by Schulthess and colleagues (1987; 1997) to enhance biological control consists of selecting for cassava with high leaf nitrogen in order to increase the size of cassava mealybugs and thereby influence a female-biased sex ratio in *A. lopezi*.

6 Summary and Perspectives

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae), is a perennial root crop native to tropical America and introduced into Africa by the Portuguese in the 1600s, and after into Asia. Since that time, cassava has constituted a major food crop for more than 500 million people in the tropical countries of Africa, Asia and Latin America. It is cultivated mainly for its starchy storage roots, but also for its leaves.

Cassava can endure several months of drought during its seasonal cycle. This is accomplished by a reduction in the plant's evaporative surface and by enhancement of the efficiency of water use through stomatal closure. Resistance to drought is further achieved by increasing the concentration of cell metabolites (sucrose, free amino acids and organic acids levels) to maintain the osmotic pressure of the cells. These mechanisms of stress avoidance are extremely effective in cassava, explaining the plant's ability to withstand prolonged periods of drought. The photosynthetic CO_2 assimilation pathway of cassava has been a matter of debate. Early work classified cassava as a C3-C4 intermediate. Recently, it was demonstrated that the plant displayed C3 photosynthesis. In conclusion, cassava does not possess a particular photosynthetic metabolism that enables the plant to resist drought, but it does exhibit efficient mechanisms of drought resistance generally displayed by the C3 plants.

In Africa and South America, outbreaks of natural populations of phloem feeder insects, occur on cassava every year during the dry season causing severe damages. The insects are two oligophagous mealybugs species, *Phenacoccus manihoti* Matile-Ferrero and *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae), displaying a typical phloem-feeding behaviour and a predominance of extracellular pathways of the stylets. Several methodologies (microscopic observations, physiological studies…) have been used to study the mealybug–plant interactions. The third trophic level relative to natural enemies (parasitoids and predators) has been also considered.

The cassava mealybug, *Phenacoccus manihoti,* possesses sensilla on its antennae that can detect chemicals released by the plant by olfaction and contact. The presence of contact and olfactory chemoreceptors on the labial tip suggests that by tapping the

cassava leaf, information about the chemical nature of the leaf surface can be gathered. Linamarin, a cyanogenic compound present on cassava phylloplane, is involved in host-plant recognition and acceptance by the mealybug mobile stages (crawlers). Electropenetrography (EPG) recordings suggest a possible use of cyanides or cyanogenic compounds as allelochemicals for host recognition during the cell penetration process by *P. manihoti*.

As mentioned above the cassava mealybugs are phloemophagous insects, reaching then the phloem sap of cassava. It has been demonstrated for *P. herreni* that the delay in reaching the phloem by the stylets is less when the insect is located near a major leaf vein, explaining why the mealybugs are usually spaced in areas around a major leaf vein. In the case of *P. manihoti,* it was observed also that on less preferred host plants, it takes longer for the stylets to reach the phloem; this appears to be related to the higher levels of phenolic acids in the apoplastic compartment of leaf tissues of such plants.

During the stylets pathway process to reach the phloem sap, *P. manihoti* secretes pectinolytic salivary enzymes that facilitate intercellular stylet penetration into the host tissues by digesting the constituents of the middle lamellae and primary cell walls. An analysis of the enzymes present in the midgut of *P. herreni* was carried out, and the intestinal pH was estimated to be between pH 6.8–7.6, close to that of the phloem sap, which ranges between pH 7.2–8.5. As mealybugs are mainly phloem-feeders with a diet of simple food constituents such as free amino acids, only a few enzymes were detected in the midgut. Of 19 enzymes assayed, only alkaline phosphatase, esterase and leucine aminopeptidase activities were evident in the midgut of the insect.

It has been demonstrated that linamarin (a cyanogenic glucoside) is translocated by the phloem sap of cassava. Although this compound appears to have a limited effect on mealybug development and physiology, it plays an important role in stimulating feeding. In contrast, rutin (a flavonoid glycoside), also translocated by the phloem sap of cassava, affects the growth and the development of the insect. These results have been demonstrated with *P. manihoti*.

Phagostimulants play a very important role in cassava mealybugs and in phloem-feeding insects in general. Sucrose has been found to be a strong phagostimulant. Studies on *P. herreni* showed that certain amino acids, either alone or in combination, act synergistically with sucrose as phagostimulants. Aspartic acid, glutamic acid,

valine and alanine are important phagostimulatory factors, whereas lysine, ornithine, asparagine, methionine and histidine have a mainly nutritive function. Other amino acids such as glutamine, cysteine, tryptophan, glycine and arginine play both phagostimulant and nutritional roles. Glutamine, cysteine, tryptophan, lysine, ornithine, asparagine, glycine, methionine and arginine were found to be essential for the development of *P. herreni*. Other compounds such as vitamins, cholesteryl benzoate and oligoelements also are important constituents of the development of mealybugs.

As mentioned above, outbreaks of natural populations of cassava mealybugs occur on cassava every year during the dry season in Africa and South America. Since in drought-stressed cassava nutrients such as sucrose and amino acids are either more concentrated or better balanced, such plants are more suitable for the development and reproduction of mealybugs. This has been demonstrated with *P. herreni.* Simultaneously, it has been demonstrated with *P. manihoti* that the partial resistance of cassava (both antixenosis and antibiosis) decreases during the dry season. All the above-mentioned conditions combine to ensure that drought-stressed plants are physiologically more favourable for infestation by the cassava mealybugs, and serve to enhance mealybug infestation build-up during long dry seasons in the field.

Cassava resistance to mealybugs involves both intrinsic mechanisms (antixenosis, antibiosis and tolerance) and extrinsic mechanisms, which are related to the interactions between mealybug-infested plants and natural enemies. Antixenosis of cassava to *P. manihoti* involves chemical factors such as linamarin for initial host recognition and for phagostimulation of larvae and adults, suggesting that cassava varieties with high levels of linamarin are the most preferred by the pests.

Rutin in cassava disrupts *P. manihoti* growth and development by having an anti-nutritive effect, delaying the insect's development into the adult rather than having a toxic effect, as no toxicity was observed. This compound participates in the antibiosis resistance of cassava to *P. manihoti*, or is at least linked to an induced (defensive?) reaction of cassava towards the insect. This defensive reaction is manifested by an increase in the levels of rutin and also of total phenolic compounds (mostly flavonoids) after mealybug infestation.

All cassava varieties studied to date are tolerant to *P. manihoti* infestation. Although different levels of partial resistance (antixenosis and antibiosis) are evident, total resistance (complete absence of mealybugs) has not been found in *M. esculenta*.

Jatropha gossypiifolia (Euphorbiaceae), a species closely related to *Manihot esculenta,* has been shown recently to display total resistance to *P. herreni* by both antixenosis and antibiosis mechanisms, resulting in a 100% mortality shortly after infestation. Therefore, the use of *J. gossypiifolia* in a breeding programme might afford an opportunity for obtaining hybrid plants resistant to the cassava mealybugs.

It has been demonstrated with *P. manihoti* and its natural enemies that mealybug infested cassava plants have been shown to be a source of volatiles that attract at long distances both female parasitoids and coccinellids to the microhabitat of the herbivore. This emission of volatiles does not appear to be limited to the infested parts of the plant, but occurs systemically throughout the plant. After landing on an infested plant, the mealybug-derived chemicals such as the O-caffeoylserine from the body surface of the insect, play a role in host location for the parasitoid. This last result has been demonstrated with *P. herreni* and its associated parasitoids.

The above results on resistance mechanisms of cassava to mealybugs show clearly that different types of mechanism are involved in cassava resistance towards the insects. Current plant breeding programmes to improve the resistance of plants to insects generally consider only intrinsic resistance mechanisms but not extrinsic mechanisms (*i.e.* effects of plants on the third trophic level). Future cassava breeding programmes should consider a more holistic approach by integrating the different resistance mechanisms reported here.

After more than 30 years of intensive research on the cassava mealybugs, some questions have never been resolved, among them the reasons for the year-to-year regional fluctuations in *P. manihoti* infestations in Africa and the inefficiency of the introduced parasitoid *Apoanagyrus lopezi* in a number of ecological conditions. Both *P. manihoti* and *A. lopezi* originate from a restricted area of 100,000 km^2 in Paraguay, where the insect population is naturally well regulated by the parasitoid, but where the ecological conditions are quite different from those in Africa where both species are now distributed over an area of about 8 million km^2. The new and highly variable ecological conditions in the new area of distribution might have caused the differentiation of geographical populations of mealybugs, and as a consequence might have modified the cassava–mealybug interactions and the suitability of the mealybug to *A. lopezi,* its main natural enemy. This is likely because both the second (*P. manihoti)* and third (*A. lopezi*) trophic levels are highly specialized.

Cassava was introduced into Africa four centuries ago by the Portuguese, while the introduction of the cassava mealybug and its parasitoid occurred more recently,

35 and 20 years ago, respectively. The three trophic levels have evolved separately under different abiotic and biotic constraints. Future prospects on cassava–mealybug–natural enemies interactions should consider the population variability of the three trophic levels at both phenotypic (phenotypic plasticity) and genetic (modification of the genome under selection pressure) levels. Understanding how cassava–mealybug–natural enemy interactions evolved should help in developing a more effective strategy for integrated management of the cassava mealybugs, pests which have the potential to wreak havoc on the food security of peoples in the tropics who rely on this crop as a vital food staple.

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Appendix 1

Material and methods used in the molecular-based approach to the differentiation of cassava mealybug species (Sternorrhyncha: Pseudococcidae)

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■ **Insects analysed.** All mealybug populations were collected from cassava, *Manihot esculenta*. About 50 adult females of three mealybug species were used in these studies. Two populations of *Phenacoccus manihoti* were collected from San Lorenzo, Paraguay and Pointe Noire, Republic of Congo, and two *Phenacoccus herreni* populations were collected from Cruz das Almas, Brazil, and from the colony establish from individuals collected at CIAT, Palmira, Colombia. Adult females of *P. madeirensis* (Green) (Homoptera: Pseudococciade) were collected from cassava grown in the field at CIAT (Palmira, Colombia). All insects were kept in 70% ethanol and stored at -20°C until molecular analysis.

■ **DNA extractions.** Genomic DNA was extracted according to the method of Gilbertson and co-workers (1991). Each insect was entirely homogenized in 10 µL of the extraction buffer (50mM of EDTA (pH 8.0), 500 mM NaCl and 10 mM 2-mercaptoethanol). An additional 190 µL of extraction buffer and 20 µL of 10% aqueous SDS solution were added to the homogenate and thereafter the sample was incubated at 65°C for 10 min. Twenty (20) µL of potassium acetate solution (5 M, pH 5.5) was added and the reaction mixture centrifuged at 15,000 rpm for 10 min. The supernatant was collected, and 100 µL of absolute isopropanol was added. The sample was centrifuged (15,000 rpm, 10 min), the supernatant aspirated off and the pellet washed using 50 µL of 70% aqueous ethanol solution. After centrifugation (15,000 rpm, 5 min), the supernatant was pipetted and the pellet was dried. The sample was re-suspended in water and stored at -20°C.

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■ **RAPD PCR analysis**. The oligonucleotides H9 (5'-TGTAGCTGGG-3') and H16 (5'-TCTCAGCTGG-3')(Operon, Alameda, CA, USA) were used as primers to amplify the DNA using PCR. Reaction mixtures for PCR amplification consisted of 1.2 units of *Taq* DNA polymerase, 3 mM $MgCl_2$, 0.15 mM deoxynucleotide triphosphate, 0.8 µM primers, and 10 ng of DNA template in a final volume of 25 µL. The reactions were carried out using a programmable thermal controller (PTC-100, MJ Research, Waltham, MA, USA). The PCR parameters were 94°C for 5 min, 40°C for 2 min and 72°C for 3 min followed by 39 cycles of 94°C for 1 min, 40°C for 1.5 min and 72°C for 2 min. The PCR products were run in 1.5% agarose gels, stained with ethidium bromide and visualized under UV light.

■ ***Wolbachia* detection in *Phenacoccus* populations.** PCR was carried out with specific *Wolbachia ftsZ* gene primers (Holden *et al.*, 1993). Two kinds of PCR were performed: the classical method as described by Holden and co-workers (1993) and Heddi and co-workers (1999) and long PCR, which has been shown to improve *Wolbachia* DNA amplification (Jeyaprakash & Hoy, 2000).

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We thank Bruno Le Rü (IRD) for providing the *P. manihoti* population from Pointe Noire (Kouilou, Congo); Aristoteles Pires de Matos (EMBRAPA) for providing the *P. herreni* population from Cruz das Alma (Bahia, Brazil); and Julio Caballero (MAG) for providing the *P. manihoti* population from San Lorenzo (Central, Paraguay). We appreciate the technical assistance given by Pilar Hernandez in the morphological identification of the mealybug species. This work was supported in part by the French Ministry of Foreign Affairs.

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Appendix 2

Materials and methods used in studying digestive enzymes from the cassava mealybug *Phenacoccus herreni*

D. F. Mùnera[1], A. Valencia-Jimenez[2], D. P. Preciado[2], G. A. Ossa[2], A. C. Bellotti[3] & P.-A. Calatayud[1]

■ **Insects analysed.** A culture of *Phenacoccus herreni* was maintained on cassava (var. CMC 40) in a greenhouse at 27-33°C and L12:D12 photoperiod. About 50 adult females were collected and used for the analyses.

■ **Mealybug dissection.** Mealybug midguts were dissected in distilled water under a binocular microscope. Midguts were extracted by pulling the caudal/anal region to extract part of the rectum and the midgut, then pulling out the head (attached to the anterior part of the midgut), cutting the oesophagus and finally extracting the almost entire midgut from the posterior end. Disrupted tracts were not used. The midgut was then rinsed in distilled sterile water and stored on ice for enzymatic analysis or used directly for pH estimation.

■ **Midgut pH determination.** The pH of the insect midgut was determined according to a method modified fromValencia-Jiménez and co-workers (2000). Each midgut was incised in part and about 1µg of dried pH indicator powder was directly deposited on the incised part. Changes in colour of the pH indicator were then observed. As a control, the same procedure was used on the distilled water in which the insect was dissected. Values between pH 4.6 and 5.2 were observed, distinct from the range obtained for the midgut fluids. The estimation of the midgut pH was done by overlapping the different pH ranges of all the indicators used (Table 1).

■ **Enzymatic analysis.** A fast semi-quantitative analysis of enzymatic activities present in the mealybug midgut was first performed using the API system (BioMérieux, Marcy l'Étoile, France). As described by Rahbé and co-workers (1995), activities were detected by cleavage of a chromogenic substrate (naphthyl derivatives) dispersed dry on a porous plastic micro-cup (~ 100 µL). Enzymatic reactions were stopped by an SDS-

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Table 1
The pH indicators used for determining mid-gut pH of *P. herreni*

pH indicator	Visual transition interval
Bromophenol blue	pH 3.0 (greenish yellow) to pH 4.6 (bluish purple)
Congo red	pH 3.0 (purple) to pH 5.2 (reddish orange)
Bromocresol green	pH 3.8 (yellow) to pH 5.4 (blue)
Bromocresol purple	pH 5.2 (greenish yellow) to pH 6.8 (bluish purple)
Bromothymol blue	pH 5.8 (yellow) to pH 7.6 (blue)
Phenol red	pH 6.5 (yellow) to pH 8.0 (reddish purple)
Cresol red	pH 6.5 (yellow) to pH 8.5 (purple)
Thymol blue	pH 7.8 (yellowish green) to pH 9.5 (blue)
Phenolphthalein	pH 8.0 (colourless) to pH 10.0 (purple)

based acid Tris Buffer (Zym A) and visualized by a Fast Blue BB solution (Zym B). The level of activity was determined by comparing the intensity of colour produced with the colour scale provided with the kit (0-5, from ≤ 5 to ≥ 40 nmols of substrate released). The API-ZYM set for screening the presence of 19 enzymes was used.

Each midgut was homogenized in 65 µL distilled water (buffer pre-set with the chromogenic substrate) and centrifuged (18,000 *g*, 10 min, + 4°C). The resultant supernatant was pipetted to a micro-cup. Twenty API micro-cups (*i.e.* 19 for enzyme analysis and 1 as control) were each filled with one midgut, then incubated (37°C for 4 h) and thereafter visualized as described above. To estimate the enzyme activity coming from the midgut, the same procedure was done with the rest of the body after extracting the midgut (rest of one corresponding body per micro-cup).

The following enzyme activities were analysed: alkaline phosphatase (pH 8.5), C4 esterase, C8 esterase/lipase, C14 lipase, leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin (*N*-benzoyl-DL-arginine-2-naphthylamidase), alpha-chymotrypsin (*N*-glutaryl-phenylalanine-2-naphthylamidase), acid phosphatase (pH 5.4), naphthol-AS-BI-phosphohydrolase, alpha-galactosidase, beta-galactosidase, beta-glucuronidase, alpha-glucosidase, beta-glucosidase, *N*-acetyl-beta-glucosaminidase, alpha-mannosidase and alpha-fucosidase.

■ **Zymograms.** To confirm the presence of the three enzymes revealed in the midgut of *P. herreni* by the API system analysis (see results in Chapter 3), zymograms were carried out with a PhastSystem electrophoresis unit (Pharmacia) following the manufacturer's instructions. For each enzyme zymogram, 50 midguts were homogenized in 20 µL distilled water and centrifuged (18,000 *g*, 10 min, 4°C).

For the enzyme zymogram, the proteins present in the supernatant were separated in gradient (10-15%) native polyacrylamide gel. After separation a nitrocellulose membrane was placed upon the polyacrylamide gel to transfer the proteins from the gel to the membrane according to the procedure of Valencia-Jiménez and co-workers (2000). This was done at 37°C for 2 h in a dark and saturated humidity. The nitrocellulose membrane was then incubated with different reaction buffers and substrates at room temperature for 10 min, depending upon the enzyme analysed as follows:

60 mM borate buffer (pH 8.5) containing 10 mM $MgCl_2$ and 50 mg of alpha-naphthyl phosphate, for alkaline phosphatase;

0.05 M phosphate buffer (pH 7.5) containing 10 mg of alpha-naphthyl acetate, for esterase;

50 mM phosphate buffer (pH 6.5) containing 40 mg of L-leucyl-2-naphthylamide, for leucine aminopeptidase (leucine arylamidase).

After incubation, the nitrocellulose membrane was stained, rinsed in distilled water and the bands fixed by 50% ethanol or 3 or 7% acetic acid for alkaline phosphate, esterase or leucine aminopeptidase, respectively. The following staining solutions were used: 50 mg of Fast Blue RR in 10 mL of reaction buffer for alkaline phosphatase and esterase, 60 mg of Fast Garner GCB in 100 mL of reaction buffer for leucine aminopeptidase analysis. The pure enzymes used as positive controls were alkaline phosphatase EC 3.1.3.1 (Sigma, P 2276), esterase EC 3.1.1.1 (Sigma, E 3019) and leucine aminopeptidase EC 3.4.11.1 (Sigma, L 5658).

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Résumé

Originaire d'Amérique du Sud, le manioc, *Manihot esculenta* Crantz, est une euphorbiacée pérenne introduite en Afrique dès le XVI^e siècle par les navigateurs portugais, puis en Asie. Il constitue aujourd'hui, de par la consommation de ses racines et de ses feuilles, la culture vivrière de base de plus de 500 millions d'habitants dans les pays tropicaux.

Le manioc a la particularité de pouvoir endurer plusieurs mois de sécheresse pendant son cycle de développement, grâce à ses facultés de réduire drastiquement sa surface d'évaporation et d'utiliser efficacement l'eau résiduelle présente dans ses tissus. De plus, en cas de déficit hydrique accru, le maintien de la pression osmotique des cellules est facilité par une augmentation importante de la concentration en métabolites cellulaires, tels que le saccharose, les acides aminés libres et certains acides organiques. Certes, ces mécanismes physiologiques d'adaptation à la sécheresse sont fonctionnels chez la plupart des végétaux, mais ils s'avèrent d'une grande efficacité chez le manioc. Cette capacité de résistance a été à l'origine de discussions sur le type de photosynthèse développé par cette plante, qui ont conduit à la classer dans un type intermédiaire, C_3-C_4. Toutefois, il apparaît à la lumière des investigations des auteurs du présent ouvrage (par l'analyse foliaire de l'abondance isotopique du carbone 13 et des enzymes associées à la photosynthèse), que le manioc utilise avec efficacité toute la panoplie des mécanismes de résistance physiologique à la sécheresse connues chez les C_3 et qu'il a donc plutôt une activité photosynthétique de type C_3.

Pendant la saison sèche, le manioc souffre des pullulations de deux espèces d'insectes piqueurs-suceurs, qui sont à l'origine de pertes importantes de récolte. Il s'agit de cochenilles farineuses oligophages du genre *Phenacoccus* (Sternorrhyncha : Pseudococcidae), *Phenacoccus manihoti* Matile-Ferrero et *Phenacoccus herreni* Cox & Williams, qui manifestent un comportement d'alimentation typiquement phloémophage (*i.e.* se nourrissant principalement de la sève phloémienne ou élaborée) et qui, pour cette raison, se positionnent souvent à proximité des nervures foliaires hébergeant les vaisseaux de la sève élaborée. D'une manière intéressante, la première espèce sévit en Afrique, la seconde en Amérique du Sud. On dispose donc d'un « modèle »

pour analyser les différents aspects des interactions entre des ravageurs phloémophages et leur plante hôte, sachant que des investigations de ce type avaient été, jusqu'il y a peu, menées essentiellement sur des pucerons. Le troisième niveau trophique relatif à l'intervention des ennemis naturels tels que les prédateurs et les parasitoïdes a également été pris en compte. Ce modèle a été étudié dans un contexte d'agriculture de petites parcelles et de petits exploitants, à partir des années 1980, par des chercheurs de l'IRD alors en poste en République Populaire du Congo. Toute une palette d'approches et de méthodologies (observations en microscopie électronique, études comportementales et physiologiques, résistance variétale avec utilisation de cultivars locaux, aspect tritrophique…) a été mise en application pour progresser dans la compréhension des séquences du mécanisme de sélection de la plante par les cochenilles : choix de la plante, prise alimentaire et nutrition. L'objet du présent volume de la collection « Didactiques » est de présenter une synthèse des connaissances acquises à ce jour dans ce domaine.

Sur les antennes et sur le labium (partie inférieure de l'appareil buccal de l'insecte) de *P. manihoti* ont été observées des sensilles capables de détecter, par olfaction et contact, des composés chimiques libérés par le manioc. La présence de telles sensilles suggère que la cochenille acquiert, en tapotant la surface du végétal, des informations chimiques l'informant sur la nature de la plante sur laquelle elle se trouve. Ainsi, les jeunes stades larvaires de *P. manihoti* reconnaissent leur plante hôte grâce à la linamarine, un glycoside cyanogénique caractéristique du manioc et présent à la surface de ses feuilles. Ce composé, également présent dans les vacuoles des cellules du mésophylle, peut être également reconnu par l'insecte lorsque ses pièces buccales pénètrent les tissus de la plante ; les stylets suivent alors, principalement, un trajet intercellulaire mais ponctionnent à l'occasion des cellules mésophylliennes. Pendant cette phase, la progression des stylets est facilitée par la digestion, par les enzymes salivaires de la cochenille, des éléments constitutifs de la paroi des cellules végétales. Sur les plantes moins préférées par l'insecte, les auteurs ont pu constater que la progression des stylets dans les tissus foliaires est plus difficile. Cela est vraisemblablement dû à une concentration plus élevée de composés phénoliques intercellulaires chez ce type de plante.

En relation avec les teneurs élevées de la sève phloémienne en éléments biochimiques simples tels que les acides aminés libres, et comme chez tous les insectes s'en nourrissant (e.g. pucerons, aleurodes), on trouve peu d'enzymes digestives dans le tractus digestif des cochenilles. Ainsi, seules 3 enzymes digestives ont été mises en évidence

chez *P. herreni*, sur les dix-neuf analysées : la phosphatase alcaline, l'estérase et la leucine aminopeptidase. De plus, le pH de son tractus digestif est compris entre 6,8 et 7,6, et donc proche de celui de la sève (compris entre 7,2 et 8,5).

Plusieurs composés de la sève phloémienne jouent un rôle important dans l'alimentation des cochenilles. La linamarine stimule la prise de nourriture de *P. manihoti*. À l'inverse, la rutine, un flavonoïde glycosylé, a un effet anti-appétant qui affecte à terme le développement et la croissance de cet insecte. Le saccharose, composé majoritaire de la sève phloémienne chez les végétaux, stimule fortement la prise de nourriture chez les cochenilles farineuses du manioc. Chez *P. herreni* en particulier, il agit en synergie avec certains acides aminés libres pour assurer la phagostimulation. Ainsi, lorsqu'ils sont associés avec le saccharose, l'acide aspartique, l'acide glutamique, la valine et l'alanine sont phagostimulants alors que la lysine, l'ornithine, l'asparagine, la méthionine et l'histidine ont un rôle nutritif. D'autres acides aminés comme la glutamine, la cysteïne, le tryptophane, la glycine et l'arginine ont un rôle à la fois phagostimulant et nutritif. De plus, la glutamine, la cysteïne, le tryptophane, la lysine, l'ornithine, l'asparagine, la glycine, la méthionine ou l'arginine sont des acides aminés essentiels au développement de *P. herreni* : leur absence dans l'alimentation de l'insecte empêche son développement. D'autres composés comme certaines vitamines, le benzoate de cholestérol et certains oligo-éléments sont également essentiels au développement et à la croissance des cochenilles du manioc.

Lors de périodes de sécheresse prolongées, un processus physiologique de réponse au déficit hydrique se manifeste dans les feuilles de manioc. Les concentrations en saccharose et en acides aminés de la sève phloémienne augmentent, la rendant plus favorable au développement et à la reproduction de *P. herreni*. Simultanément, le manioc devient moins résistant aux attaques des cochenilles, comme cela a été illustré par des études au champ avec *P. manihoti* montrant que la réponse défensive de la plante à l'attaque des insectes est moins prononcée pendant ces périodes. Ainsi, toutes les conditions sont réunies pour permettre une augmentation importante des effectifs de cochenilles pendant une période de sécheresse prolongée, ce qui est effectivement fréquemment observé dans les plantations de manioc.

L'ensemble de ces résultats est discuté par les auteurs en termes de résistance du manioc à l'attaque des cochenilles farineuses. Les données existantes permettent de préciser d'abord les mécanismes intrinsèques de résistance mis en jeu au niveau de la plante (antixénose ou non-préférence, antibiose et tolérance). L'antixénose fait ainsi intervenir des facteurs chimiques, comme la linamarine, dont le rôle en tant que

composé de reconnaissance de la plante hôte et de phagostimulation a été montré chez *P. manihoti*. En contrepartie, les composés phénoliques inter-cellulaires gênent la progression des stylets dans les tissus de la plante, la rendant ainsi moins préférée par l'insecte. Du fait de son rôle anti-appétant, la rutine est plutôt impliquée dans les mécanismes d'antibiose qui perturbent le développement et la croissance de *P. manihoti*. Ce composé est lié à la manifestation d'une réaction défensive du manioc à l'infestation par les cochenilles, qui se traduit par une augmentation significative en rutine ainsi qu'en d'autres composés phénoliques (principalement des flavonoïdes) foliaires. Il est intéressant de noter que cette réaction se manifeste également lors d'infections bactériennes. En plus de l'antixénose et de l'antibiose qui sont impliqués dans la résistance du manioc aux cochenilles, toutes les variétés de manioc étudiées ont montré qu'elles étaient tolérantes à l'infestation par *P. manihoti*. Lors de récentes investigations, une plante génétiquement proche du manioc s'est avérée « totalement » résistante à *P. herreni,* causant 100 % de mortalité après seulement 48 heures d'infestation. Il s'agit de *Jatropha gossypiifolia* (Euphorbiaceae). Sa toxicité est due à la présence d'une protéine toxique et permet d'envisager un programme de sélection variétale en créant par exemple des hybrides totalement résistants à la cochenille.

D'une manière intéressante, les travaux menés par les auteurs montrent l'intervention de mécanismes extrinsèques de résistance. Lorsqu'il est attaqué par les cochenilles, le manioc émet des substances chimiques volatiles qui attirent à longue distance les ennemis naturels de ces insectes que sont les parasitoïdes et les coccinelles. Ces auxiliaires viennent alors, en quelque sorte, en aide à la plante. Les émissions de volatiles ne se limitent pas aux parties infestées mais proviennent de la plante entière. Ce phénomène a été mis en évidence dans le cas de la cochenille *P. manihoti*, à la fois vis-à-vis du parasitoïde *Apoanagyrus (Epidinocarsis) lopezi* De Santis (Hymenoptera : Encyrtidae), parasitoïde introduit d'Amérique du Sud pour le contrôle de *P. manihoti* en Afrique dans le cadre d'une opération de lutte biologique, et de la coccinelle indigène *Exochomus flaviventris* Mader (Coleoptera : Coccinellidae).

Au contact de la cochenille, les parasitoïdes identifient leurs hôtes. Ainsi, la présence d'O-caféoylsérine à la surface du corps de *P. herreni* informe les parasitoïdes *Aenasius vexans* Kerrich et *Acerophagus coccoi*s Smith (Hymenoptera : Encyrtidae) sur l'identité de l'hôte.

À l'évidence, le manioc a développé différents mécanismes de résistance vis-à-vis des cochenilles farineuses. Or, force est de constater que la plupart des travaux actuels de

sélection variétale ne s'intéressent qu'aux mécanismes de résistance intrinsèques et négligent les mécanismes extrinsèques, comme l'effet de la plante sur le 3e niveau trophique. Ainsi les auteurs ont pu montrer que l'utilisation de variétés de manioc présentant un mécanisme d'antibiose trop prononcé vis-à-vis de *P. manihoti* défavorisait le parasitisme par *A. lopezi*. Les résultats présentés par les auteurs montrent l'intérêt qu'il y a à mettre en place des programmes d'amélioration variétale du manioc en suivant une approche plus holistique prenant en compte les différents types de résistance, intrinsèque et extrinsèque.

Si les recherches menées pendant plus de trente ans sur les cochenilles farineuses du manioc ont permis de préciser certains aspects des interactions entre ces déprédateurs et leurs ennemis naturels, plusieurs questions restent toujours sans réponse. Ainsi, les raisons des importantes fluctuations interannuelles d'effectifs de *P. manihoti* d'une région à l'autre de l'Afrique demeurent toujours inexpliquées, de même que l'inefficacité du parasitoïde *Apoanagyrus lopezi* dans certaines situations écologiques. Ces deux insectes sont originaires d'une région limitée de quelques centaines de milliers de km^2 du Paraguay, où les populations de la cochenille sont naturellement contrôlées par le parasitoïde. En Afrique, les deux espèces sont présentes sur près de 8 millions de km^2 et sont donc confrontées à des conditions écologiques beaucoup plus variables que celles de leur région d'origine. Il est possible que cette situation ait conduit à la différenciation de populations locales de cochenilles, se manifestant par une modification des interactions de l'insecte avec sa plante hôte et une moins bonne adéquation de celle-ci avec ses principaux ennemis naturels. Cela apparaît d'autant plus vraisemblable que le manioc a été introduit en Afrique il y a près de quatre siècles alors que l'introduction de la cochenille et celle de son parasitoïde sont intervenues récemment, il y a trente-cinq et vingt ans respectivement. Les trois niveaux trophiques ont ainsi évolué séparément. Comprendre comment les interactions manioc-cochenille-ennemis naturels ont évolué devrait aider à mieux gérer la lutte intégrée contre les cochenilles du manioc.

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Most basic information on plant-mealybug interactions during the last decade has come from research on the cassava *Manihot esculenta* Crantz (Euphorbiaceae) system with two mealybug species, namely *Phenacoccus manihoti* Matile-Ferrero and *Phenacoccus herreni* Cox and Williams (Sternorrhyncha: Pseudococcidae). Both these insects cause severe damage to cassava in Africa and South America, respectively.

This book reviews these interactions (plant selection by the insects, nutritional requirements, influence of the climate, intrinsic and extrinsic plant-defence mechanisms). It should be useful for entomologists, crop scientists, agronomists and ecologists as well as for teachers and students.

Au cours des dernières décennies, la plupart des recherches sur les interactions plantes-cochenilles farineuses ont porté sur le modèle manioc-cochenilles (Manihot esculenta *Crantz, Euphorbiaceae*/Phenacoccus manihoti *Matile-Ferrero et* Phenacoccus herreni *Cox et Williams, Sternorrhyncha, Pseudococcidae). Ces insectes peuvent être respectivement responsables de pertes de récoltes importantes dans les parcelles de manioc en Afrique et en Amérique du Sud.*

Cet ouvrage présente ces interactions ainsi que les facteurs qui les influencent : mécanismes de sélection de la plante par les insectes, identification des éléments nutritifs de la plante pour le développement des insectes, mécanismes de défense intrinsèques et extrinsèques de la plante aux insectes et influence du climat sur ces interactions. Il s'adresse aux entomologistes, améliorateurs de plantes, agronomes et écologistes ainsi qu'aux enseignants et étudiants.

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