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Influence of parasitism by encyrtid parasitoids on the feeding behaviour of the cassava mealybug *Phenacoccus herreni*

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Abstract

Three encyrtid parasitoids *Apoanagyrus (Epidinocarsis) diversicornis, Aenasius vexans*, and *Acerophagus coccois* (Hymenoptera: Encyrtidae) are used to control the cassava mealybug *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae), an important pest of cassava in South America. The influence of parasitism on the feeding behaviour of mealybugs was studied by observing honeydew secretion and by the electrical penetration graph technique (EPG, DC-system). Honeydew secretions were observed after parasitism until mummy transformation. No strong EPG parameter differences were found between parasitised and control insects. All results indicated that parasitised mealybugs keep feeding on the phloem sap after parasitism until mummy transformation. The main influence of parasitism on EPG parameters is the appearance of a new pattern resembling the E2 pattern at the extracellular level and labelled H. This pattern was also produced with control insects located on an unfavourable feeding site and could be associated with a stress response. It might be related to the still unclear E(c) pattern of aphids. The relationship of H to stylet activities is discussed.

Introduction

In South America (especially in Northeast Brazil), the cassava mealybug *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae) is an important pest of cassava, *Manihot esculenta* Crantz (Euphorbiaceae), particularly during drought periods when the insect population increases (Bellotti et al., 1983; Noronha, 1990). To control this mealybug, three encyrtid koinobiont parasitoids *Apoanagyrus (Epidinocarsis) diversicornis* Howard, *Aenasius vexans* Kerrich and *Acerophagus coccois* Smith (Hymenoptera: Encyrtidae) are being studied at the International Centre for Tropical Agriculture (CIAT, Cali, Colombia). They were released in semiarid areas of the Brazilian states of Bahia and Pernambuco in 1994 and 1995 (Smith & Bellotti, 1996; Bertschy, 1998). Despite the presence of cassava mealybug parasitoids, increases in pest populations are often reported during long dry seasons (Bellotti, pers. obs.). These increases could be due to biochemical changes in the cassava leaves, induced by water deficiency, which has a positive effect on pest development and a negative effect on parasitoid development. In order to identify how some changes in the nutrition of *P. herreni* could influence parasitoid development, it was first necessary to study the feeding behaviour of parasitised mealybugs.

For koinobiont parasitoids (Askew & Shaw, 1986), whose larvae develop in a host that continues to feed and grow after having been parasitised, nutritional interactions with the host insect play an important role in insect parasitoid development (Vinson & Iwantsch, 1980) because the parasitoid larvae compete with the host tissues for available nutrients (Thompson, 1982; Cloutier, 1986), thereby affecting the host's feeding and food utilisation efficiency (Cloutier & Mackauer, 1979). Therefore, the type of food hosts consume will influence the development of their parasitoids (Harvey et al., 1995; Hofstetter & Raffa, 1997). Some studies have demonstrated that parasitism induces physiologi-

cal alterations of their hosts (Pennachio et al., 1994, 1995; Islam et al., 1997); in contrast, no study, to our knowledge, has clearly reported on the influence of parasitism on the host's feeding behaviour.

Phenacoccus herreni is a phloemophagous insect (Polanía et al., 1999). Assessing the pathways followed by stylets through leaf tissue can be done using the electrical penetration graph technique (EPG, DC-system). This technique was developed to monitor the probing activities by aphids (Tjallingii, 1978) and has since been used to study probing activity of the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Sternorrhyncha: Pseudococcidae) (Calatayud et al., 1994). The output EPG waveforms are determined by the insect's behaviour and position of the stylet tip in the leaf tissue (Tjallingii, 1988).

The purpose of this work was to study the influence of parasitism on the feeding behaviour of *P. herreni* using the EPG, DC-system. The parasitoid species used were *A. diversicornis, A. vexans* and *A. coccois*. As EPG recording is technically difficult to carry out continuously over several days due to moult formation by the mealybugs and limited computer storage capacity (see Material & Methods), the duration of mealybug feeding activity after parasitism was first estimated through observations on honeydew secretion to determine how long before mummy transformation the insects showed feeding activity. Just before this, parasitised insects were recorded by EPG, assuming that these EPGs are characteristic of the feeding behaviour of parasitised mealybugs.

Observations of *P. herreni* populations on plants – regardless of their developmental stage, sex and parasitism – show a distribution mainly around major leaf veins. Given that mealybugs are mobile and that it is impossible to wire them without removing/disturbing them, standardisation of the EPG method for parasitised mealybugs had to be worked out. The effect of insect location (near or far from a major leaf vein) on the EPG parameters was first studied to determine which leaf location would be better to observe the influence of parasitism on host feeding behaviour.

Materials and methods

Plants and insects. One-month-old cassava plants (cv. CMC 40), obtained from cuttings, were grown in individual plastic pots (30 × 22 cm) containing peat and sand in a glasshouse at 28–35 °C and L12:D12 photoperiod. A culture of *P. herreni* was maintained on cassava (cv. CMC 40) in a glasshouse at 27–33 °C and L12:D12 photoperiod. Females at third instar, the preferred stage for parasitism (Van Driesche et al., 1987; Bertschy, 1998), and adult females from the colony were used for the experiments on the influence on EPG parameters of parasitism on the feeding behaviour of *P. herreni* and mealybug location on the leaf, respectively.

The parasitoids *A. diversicornis* (asexual strain), *A. vexans* and *A. coccois* (Hymenoptera: Encyrtidae) were reared continuously on mealybug-infested cassava plants (cv. CMC 40) in a glasshouse at CIAT at 27–33 °C and L12:D12 photoperiod. Emerging adults were collected for each parasitoid species, placed in transparent polystyrene tubes (15 × 1.5 cm) for fourty eight hours and fed with honey droplets. Then twenty female mealybugs at third instar were exposed to five female parasitoids and observed for parasitism. Only parasitised mealybugs were selected and used for the experiments.

EPG recording. Stylet penetration was monitored by an EPG-DC system (Tjallingii, 1988) with an input resistance of 10^9 Ohm (Model Giga 4 DACQ 95, The Netherlands). Mealybugs were placed in a plastic petri dish for 1–2 min. A gold wire (2–3 cm, 17.5 μm) was then fixed to the dorsum of the insect with a water-based silver paint. The insect was connected to the amplifier before being placed on a leaf; experiments were carried out in a Faraday cage at a temperature of 30 °C. For EPG recordings, a MacADIOS 8ain data acquisition system (with a quickBASIC software and digitising card) was used, converting and storing (−5+5V) EPG signals at a 100-Hz sampling rate. The stored signals were then transferred to a Macintosh-based EPG analysis software (MacStylet), allowing off-line analysis and statistical treatment of the recording (Febvay et al., 1996).

To study the influence on EPG parameters of mealybug location on the leaf, adult females from the colony were recorded near or far from a major leaf vein. To study the influence of parasitism on the feeding behaviour of *P. herreni*, the parasitised third instar females were transferred to the lower side of a cassava leaf and enclosed in an adapted plastic petri dish. Nonparasitised mealybugs (third instar, used as control) were placed under the same conditions. Daily, a portion of thin-layer plate of silica gel 60 (Merck) was placed under each individual for eight hours and then revealed by ninhydrine reagent (0.2% in N-butanol) to observe honeydew excretion. The du-

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This same experiment was duplicated, and before mummy transformation the insects were recorded. Both parasitised and nonparasitised mealybugs under the same experimental conditions were at adult stage for EPG recordings. Only EPGs done on parasitised mealybugs whose mummies showed adult parasitoid emergence were considered. For each treatment EPG recordings were done for a total of eight hours and ten females.

EPGs. The existing background work on the cassava mealybug *P. manihoti* (Calatayud et al., 1994) was used as a reference for correlating EPG patterns with various components of the stylet penetration process. Different electrical waveform patterns can be distinguished on the basis of their duration, voltage level, relative amplitude and electrical origin. The electrical origin indicates whether a pattern element is due to resistance fluctuation (R) and/or electromotive forces (emf) (Tjallingii, 1985). Apart from the aforementioned characteristics, peak and wave-shaped fluctuations could be distinguished in some patterns and are indicated here as 'peaks' and 'waves', respectively. For spectral analysis of some EPG patterns, the software's Fourier transform algorithm function (SAS version 6.12) was used to trace the spectrum of a given digitised signal slice.

Number and total duration of EPG parameters were noted, and the percentages of total duration of EPG parameters were calculated. Time to the first sustained phloem phases in a probe, used as an indicator of phloem finding, was noted.

Statistical analyses. Tests were performed with Statview software (Abacus Concept, USA). All variables showing homoscedasticity and data normality by F test and Kolmogorov–Smirnov method, respectively, were analysed by Student's t-test for EPG parameters in Table 1 and subjected to Fisher's PLSD test following the ANOVA for EPG parameters in Table 3. For all other variables (parameters 3, 6, 8, 9 and 12 in Table 1; T1 and T2 in Table 2; parameters 6, 8 and 12 in Table 3), nonparametric Kurskal–Wallis and Mann–Whitney U tests were applied. Before running the ANOVA, the data of parameters 3 and 9 in Table 3 were normalised by $\log(X + 1)$ transformation.

Results and discussion

Influence of mealybug location on leaf on EPG parameters. *Phenacoccus herreni* EPGs showed strong similarity to waveforms produced by *P. manihoti* (Calatayud et al., 1994). Therefore it was decided to label them similarly (Figure 1), and by analogy it was assumed that *P. herreni* EPG patterns were correlated with stylet activities as follows:

- Pattern C is related to extracellular stylet path activities, mainly stylet piercing and salivary sheath formation (Figures 1a and 1b).
- Pattern pd is related to a short intracellular puncture during the pathway (sudden drops in the voltage level maintained for about 12 s) (Figures 1a and 1b). Two subpatterns could be distinguished within pd (Figure 1b): pd1 with peaks at 1–2 Hz due to emf and pd2 with small irregular waves due to conductance as well as to emf (Calatayud et al., 1994).
- Pattern E represents a sieve-element puncture with sustained sap ingestion (Figure 1a). Honeydew secretions were always observed during this pattern. Two subpatterns could be distinguished within E: E2I (Figure 1c) with peaks at 1–3 Hz due to emf and E2II (Figure 1d) with small irregular waves due to conductance and emf (Calatayud et al., 1994). Although E2I was predominant, and sometimes occurred alone, frequent alternations with E2II occurred.

Table 1 presents the different EPG parameters obtained for two insect locations (near or far from a major leaf vein). The main effect of insect location on EPG parameters was the appearance of an E2-like pattern at the extracellular level, labelled H, when mealybugs were recorded far from a major leaf vein (Figure 2 and Table 1); it might be related to the still unclear E(c) pattern of aphids (Tjallingii, 1990). This H pattern was observed in only seven of the ten individuals recorded and no mealybug located near a major leaf vein showed this pattern.

Table 1. Means and standard errors ($n = 10$) of EPG parameters in association with two leaf locations (near a major leaf vein (v-insect) or not (insect)). Different letters in the same row indicate significance ($P < 0.05$) derived from the Student's t-test. The significance test for parameters 3, 6, 8, 9 and 12 was carried out using the Mann–Whitney U method

EPG parameters	v-insect	insect
1. No. of nonpenetrations (np)	2.8 ± 0.5 a	5.1 ± 0.8 b
2. No. of cell punctures (pd)	78.9 ± 14.2 a	107.9 ± 14.0 a
3. Duration of total np (min)	26.4 ± 3.9 a	89.9 ± 25.5 b
4. Duration of total pd (min)	16.0 ± 3.0 a	23.3 ± 2.6 a
5. Duration of total cell-wall activities (C) (min)	166.3 ± 34.3 a	240.6 ± 20.2 a
6. Duration of total phloem phases (E) (min)	271.3 ± 39.5 b	30.7 ± 19.3 a
7. Duration of total H (min)	0	95.5 ± 36.2
8. Time to first sustained E in probe (min)	30.9 ± 8.4 a	123.6 ± 33.8 b
9. Percentage of np	5.5 ± 0.8 a	18.7 ± 5.1 b
10. Percentage of pd	3.3 ± 0.6 a	4.9 ± 0.5 a
11. Percentage of C	34.6 ± 7.1 a	50.2 ± 4.2 a
12. Percentage of E	56.6 ± 8.2 b	6.4 ± 4.0 a
13. Percentage of H	0	19.8 ± 7.5

Two subpatterns could be distinguished within H: H1 showing peaks at about 1–3 Hz and H2 with small irregular waves (Figure 2b). Although H1 was predominant and sometimes occurred alone, frequent alternations with H2 occurred. The H pattern occurred frequently in the first hour of a probe (Figure 2a) or sometimes gradually after a pattern E (data not shown), but no honeydew secretion was observed during this pattern. When the plant voltage was changed from the usual positive adjustment (Tjallingii, 1985) to about 0 V and then to negative, the H1 peaks were not inverted with voltage adjustment (Figure 2c), demonstrating that these peaks were due to emf and not to conductance fluctuation in the insect-plant circuit. This same observation was made on peaks of subpattern E2I at an intracellular voltage level (Calatayud et al., 1994). Voltage adjustments were also made within H2 and revealed that the irregular waves were due to conductance and emf (adjustments not shown).

Spectral analyses of subpatterns H1 and E2I showed similarities (Figure 3). In fact, approximately 60% of the H1 signal periodicity was around 1.56 and 3.22 Hz (Figure 3a); and 59% of the E2I signal periodicity was around 1.46 and 2.83 Hz (Figure 3b), indicating that these two signals are represented mainly by peaks at about 1–3 Hz. Given that H is at the extracellular level and that no honeydew secretion was observed during this pattern, it cannot be related to phloem ingestion.

Table 1 also shows that insect location influenced the phloem-finding process. The total duration and percentage of phloem phases (E) were significantly higher when the insect was located near a major leaf vein. In contrast, the time to the first sustained E in probe (indicator of phloem finding) was significantly lower. Moreover, the nonprobing parameters (e.g., no., total duration and percentage of nonpenetration) could be related to phloem-finding difficulties because several nonprobing events occurred, showing absence of a phloem phase (data not shown). These parameters were lower when the insect was located near a major leaf vein.

In conclusion, without introducing other influencing factors, insect location on a leaf strongly influences the EPG parameters. It appears important for EPG studies with mobile insects to respect the natural insect location to prevent artifactual variations of EPG parameters. For *P. herreni*, feeding near a major leaf vein facilitates the phloem-finding process. For the remaining EPG studies, only insects near a major leaf vein were recorded.

Influence of parasitism on the feeding behaviour of P. herreni. The duration of mealybug feeding after parasitism (T1) was significantly lower when the insect was parasitised by *A. coccois* (Table 2). The time between parasitism and appearance of mummies (T2) was significantly longer in mealybugs parasitised by *A. diversicornis*.

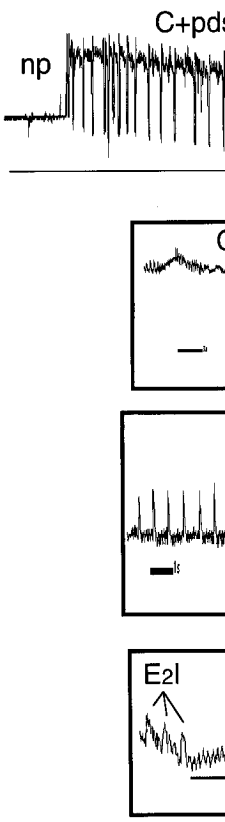

Figure 1. P. herreni EPG
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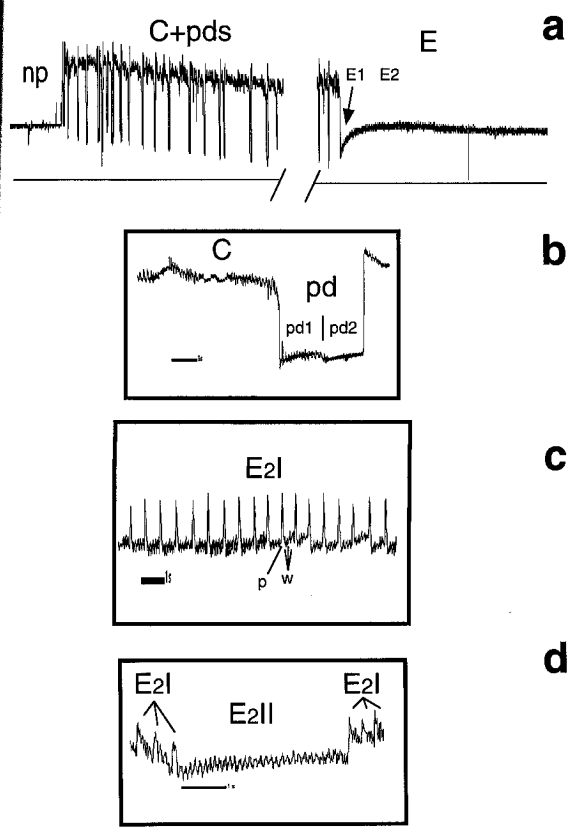

Figure 1. P. herreni EPG patterns. (a) Model 3-h recording, showing a sequence of cell-wall activities and cell punctures (C+pds) and phloem cell ingestion (E) with its two subpatterns E1 and E2 (np: nonpenetration). (b) Zooming in around an intracellular puncture, showing patterns C and pd with their two subpatterns pd1 and pd2. (c) Zooming in E2, showing subpattern E2I (p=peaks, w=waves). (d) Zooming in E2, showing subpattern E2II enclosed by E2I.

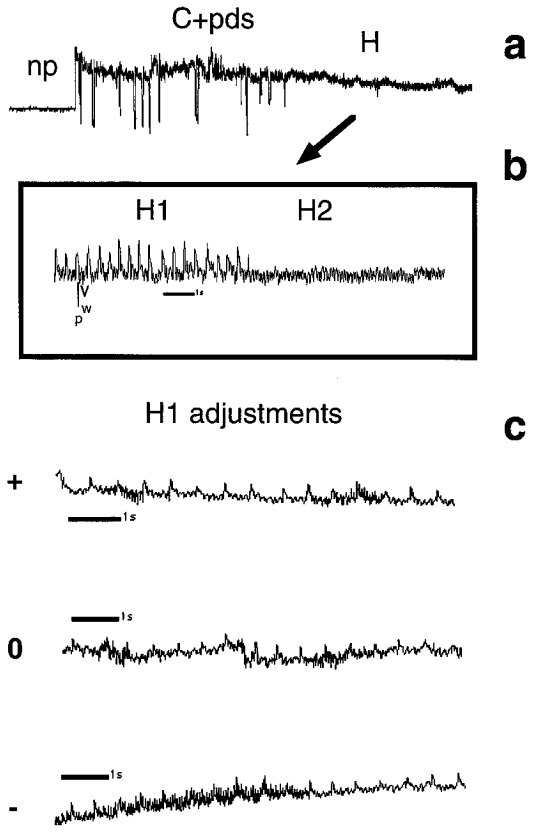

Figure 2. Details of pattern H from *P. herreni*. (a) Model 1-h recording, showing a sequence of cell-wall activities and cell punctures (C+pds) and the similar E2 pattern labelled H because of extracellular level. (b) Zooming in H showing subpatterns H1 (p=peaks, w=waves) and H2. (c) Voltage adjustements within H1 pattern, resolving the emf nature of the peak elements.

Furthermore, Table 2 indicates that parasitised mealybugs fed on sap and potentially on phloem sap (strong positive coloration of the honeydew by ninhydrin, indicating the presence of free amino acids) until approximately one day before mummification for each parasitoid species. During and after the mummy transformation process, the host ceased its feeding as no honeydew secretion was observed. For the following EPG studies, parasitised mealybugs were recorded two days before the appearance of mummies to avoid risking cessation of their feeding activity due to the mummification process (i.e., eight days after parasitism when mealybugs were exposed to *A. diversicornis* and *A. vexans* and six days after parasitism when they were exposed to *A. coccois*). Even under these conditions, some insects showed absence of stylet activities by EPG due to the mummification process (data not shown).

Table 2. Means and standard errors of total duration of mealybug feeding after parasitism (T1) and duration between parasitism and appearance of mummy (T2) for the three parasitoid species. Different letters in the same row indicate significance ($P < 0.05$) derived from the Mann–Whitney U method, following the Kruskall-Wallis test (n = no. of replications)

Durations (days)	*A. diversicornis* $n = 28$	*A. vexans* $n = 49$	*A. coccois* $n = 27$
T1	9.9 ± 0.2 b	8.9 ± 0.2 b	7.5 ± 0.1 a
T2	11.0 ± 0.3 b	10.0 ± 0.2 a	8.6 ± 0.2 a

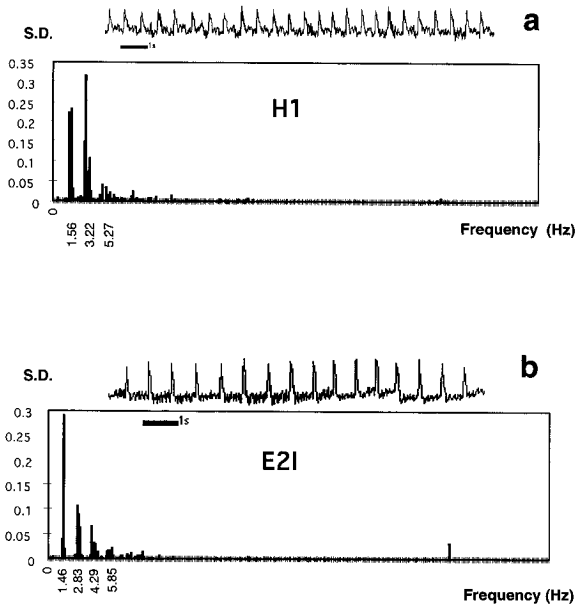

Figure 3. Spectral analysis of the different EPG patterns (Spectral Density estimate (S.D.) by frequency in Hz). (a) Frequency spectrum of subpattern H1 within H. (b) Frequency spectrum of subpattern E2I within E2.

Table 3 presents different EPG parameters obtained with *P. herreni* parasitised by three species of parasitoids, respectively. It appears that the parasitism had no strong influence on the EPG parameters (no statistical differences were found at the 5% level). Nevertheless, at the 10% level the duration of total phloem phases (E) and the percent E were significantly lower with parasitised mealybugs. In contrast, the time to the first sustained E in the probe was higher. These results could be related to certain phloem-finding difficulties or the lowest feeding activity among parasitised mealybugs. In fact, the endoparasite growing inside the host body could induce a sort of pathological weariness, causing the host to display less activity in finding phloem sap. This phenomenon is more evident when *P. herreni* is parasitised by the gregarious parasitoid *A. coccois*, [permitting larger numbers of progeny per host (Van Driesche et al., 1987)], where duration of total phloem phases (E) and the percent E were lower.

The main influence of parasitism on the EPG parameters was the appearance of the same pattern H found when the insect was located far from a major leaf vein. This pattern was not observed in all parasitised mealybugs; but nine, six, and five individuals of the ten mealybugs recorded parasitised by *A. diversicornis, A. vexans*, and *A. coccois* respectively, showed H. No control insect showed this pattern.

As H was observed when insects were parasitised and when they were in a leaf location unfavourable for reaching phloem sap, this may indicate that H is related to an insect 'stress' phenomenon. In aphids the E(c) pattern occurred almost exclusively on nonhost plants (Tjallingii, 1986). To confirm the fact that the stylet position during H pattern is extracellular, microscopic observations could be useful. The classical stylectomy cannot be applied to *P. herreni* because it is impossible to view the labium protecting the stylets when the insect is rearing on the plant. The technique developed by Calatayud et al. (1996b) to immobilise a mealybug on plants using a high-frequency microcautery unit worked, but the process of obtaining the samples for microscopy removed the insect from the leaf, mainly because the stylets are not deeply inserted in the leaf tissues, supporting the hypothesis of the extracellular location of the stylets during this pattern.

Some arguments could be made in favour of the possible relationship of H to both salivation and ingestion activities. Like pd and E2, H is composed of two subpatterns. The subpatterns of pd and E2 show some similarities, by analogy, with stylet activities in aphids:

- The first period in the pd signal, called pd1 (Figure 1b), frequently began at the extracellular level before the sudden drop in voltage level. This pd1 pattern (emf origin) could be related to salivation into the protoplast as shown by the first period in the pd signal of aphids; while the second phase, pd2 (conductance as well as emf origin) could be related to ingestion of protoplasm as shown by the last period in the pd signal of aphids (Martin et al., 1997).
- The E2I peaks (emf origin) could coincide with muscular activity of the salivary pump as shown by the E peaks in aphids (Tjallingii, 1978), in which case E2I could be related to salivation into a sieve element ; while the E2II pattern (conductance and emf origin) could be more related to ingestion activities.

Although these similarities need further investigation to verify any suggestion to a similar insect activity, the dichotomy of pd, E2, and H patterns suggests similar activity phases. Consequently, H1 (emf origin, Figure 2c) could be related to salivation into apoplast, while H2 (conductance and emf origin) could relate more to apoplast fluid ingestion. In previous work Vargas et al. (1989) showed that *P. herreni* extracts the

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9. Percentage of np
10. Percentage of pc
11. Percentage of C
12. Percentage of E
13. Percentage of H

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Table 3. Means and standard errors (n = 10) of EPG parameters in association with parasitism or nonparasitism. Different letters in the same row indicate significance (P<0.1) derived from the Fisher's PLSD test following the ANOVA (for parameters 4, 5, 10 and 11 no letter was given because no significant difference was found by ANOVA). The significance test (P<0.1) for parameters 6, 8 and 12 was carried out using the Mann-Whitney U method

EPG parameters	Nonparasitised	Parasitised by *A. diversicornis*	Parasitised by *A. vexans*	Parasitised by *A. coccois*
1. No. of nonpenetrations (np)	2.5 ± 0.3 b	2.3 ± 0.4 b	1.8 ± 0.4 ab	1.4 ± 0.3 a
2. No. of cell punctures (pd)	73.3 ± 13.6 b	54.3 ± 16.6 ab	38.5 ± 9.3 a	41.6 ± 8.0 a
3. Duration of total np (min)	25.3 ± 4.4 a	62.4 ± 16.0 ab	69.2 ± 25.0 ab	70.9 ± 22.7 b
4. Duration of total pd (min)	13.5 ± 2.8	11.0 ± 3.3	7.4 ± 2.3	9.3 ± 1.7
5. Duration of total cell-wall activities (C) (min)	145.0 ± 31.5	151.0 ± 37.8	123.5 ± 23.6	170.0 ± 26.8
6. Duration of total phloem phases (E) (min)	296.2 ± 37.7 b	101.0 ± 47.8 a	137.4 ± 36.4 a	97.0 ± 23.4 a
7. Duration of total H (min)	0	154.6 ± 46.2	142.5 ± 43.3	132.8 ± 44.2
8. Time to first sustained E in probe (min)	43.8 ± 11.2 a	84.4 ± 21.3 b	103.7 ± 28.4 b	87.9 ± 21.5 b
9. Percentage of np	5.3 ± 0.9 a	13.0 ± 3.3 ab	14.4 ± 5.2 ab	14.8 ± 4.7 b
10. Percentage of pd	2.8 ± 0.6	2.3 ± 0.7	1.6 ± 0.5	1.9 ± 0.4
11. Percentage of C	30.2 ± 6.6	31.5 ± 7.9	25.7 ± 4.9	35.4 ± 5.6
12. Percentage of E	61.7 ± 7.8 b	21.0 ± 10.0 a	28.6 ± 7.6 a	20.2 ± 4.9 a
13. Percentage of H	0	32.2 ± 9.6	29.7 ± 9.0	27.7 ± 9.3

calcium from cassava leaves, causing them to curl. It is well known that the cell wall contains a large amount of calcium linked to pectic substances. As reported for *P. manihoti* by Calatayud et al. (1996a), *P. herreni* could also secrete pectinolytic enzymes involved in the degradation of middle lamellae and primary cell walls to facilitate ingressing the stylets within host tissues. Moreover, as *P. herreni* extracts calcium from the leaves, the insect may also ingest calcium from apoplast fluid. Based on the foregoing, we suggest that H1 could be related to salivary activity and H2 to apoplastic fluid ingestion.

Conclusions

The feeding behaviour of parasitised mealybugs is to continue feeding on the phloem sap before mummy transformation. In fact, honeydew secretions were observed until mummy transformation after parasitism and no strong EPG parameter differences were found between parasitised and control insects (Table 3). Moreover, when adult females are parasitised, ovisacs with viable eggs were frequently observed (data not shown). All these results confirm that the species used in this study are koinobiont parasitoids, whose larvae develop in a host that continues to feed and grow after having been parasitised. Therefore, any changes in phloem sap biochemistry may influence not only the biological performances of the host but also those of the endoparasite larvae.

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Entomologia Experiment

Factors affecting *Drosophila simul*

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Abstract

In this study we investig quality in *Drosophila sim* yield, and (3) ability to a yield, but not on PCR su the lowest were from spe DNA, which translated i most dramatic decrease i and 291 bp fragments. Na regardless of naphthalene

Introduction

As more efficient metho ing DNA sequence, many molecular analyses into t fortunately, not all specir quality for molecular an standing of the paramete tion of DNA in stored spe of specimens could be u et al., 1999).

The purpose of this four killing methods and fect DNA quality. Thes cause they are traditiona entists. We define qualit of extracted DNA, (2) ex to amplify from four tar helped identify potentia DNA. Post et al. (1993) served in liquid nitrogen DNA than those preserv