

## A new technique to immobilize an aphid or a mealybug on plants using a high-frequency microcautery unit

P. A. Calatayud<sup>1</sup>, C. Nardon<sup>2</sup> & Y. Rahbé<sup>2</sup>

<sup>1</sup>ORSTOM, Laboratoire d'Entomologie, Parc Scientifique Agropolis II, 34397 Montpellier Cedex 5, France

<sup>2</sup>INRA-INSA, Laboratoire de Biologie Appliquée 406, 20 Avenue Albert Einstein, 69621 Villeurbanne Cedex, France

Accepted: November 7, 1995

**Key words:** *Macrosiphum albifrons*, *Phenacoccus manihoti*, electrical penetration graphs, stylet pathways, stylectomy, phloem sap

### Introduction

Light microscopy is the oldest tool used to describe stylet pathways of homopterous insects within their host plant. Since 1960, electrical recording of penetration behaviour by aphids has been introduced by McLean & Kinsey (1967) and modified from an AC to a DC device called the electrical penetration graph technique (EPG) by Tjallingii (1978). EPG, especially when combined with microscopy, is unique in describing the details of penetration activity, and in studying the feeding behaviour of phloem- or xylem-feeding insects.

Difficulties in linking wave-forms obtained by EPG and localisation of the stylets in plant tissues by microscopy are mainly due to problems in immobilizing the insect. We describe a simple technique with a modified use of a high-frequency (HF) microcautery unit that seems useful both for stylet immobility and, in some cases, for phloem sap collection.

### Materials and methods

As in EPG experiments, a gold wire (2–3 cm, Ø17.5 µm) was fixed on the dorsum of the insect with a water-based silver paint. Then, the gold wire was glued to a copper wire which was plugged into the amplifier 'probe' of the EPG-device (for an eventual recording of an EPG trace), or was directly connected to the 'injection needle' of the microcautery 'probe' (connected to the HF microcautery unit (Syntech), see Figure 1a). After a few hours (time necessary for reaching and

feeding on phloem, especially for the mealybug) the HF pulse was completed.

We applied this technique to *Macrosiphum albifrons* (Aphididae) on lupine *Lupinus albus* cv. Lublanc (Fabaceae) and *Phenacoccus manihoti* (Pseudococcidae) on cassava *Manihot esculenta* cv. Fetonegbodgi (Euphorbiaceae).

Light microscopy was used to reveal alterations in tissue after administering the HF pulse. In the case of stylet amputation after the HF pulse, exudates were collected for chemical analysis (with a microcapillary), and volumes were estimated by weighing (1 µg accuracy). As for previous stylectomy analyses (Rahbé *et al.*, 1990), sugars were identified and measured by a gas chromatographic method, and amino acids were assayed by ion-exchange and ninhydrin detection.

### Results and discussion

With an appropriate power adjustment, depending on the insect species (i.e. 15 W and 12 W during 0.6 s for *M. albifrons* and *P. manihoti* respectively), the HF pulse caused the death of the insect and resulted in the complete immobility of the stylets. Light microscopy observations revealed no visible change in plant tissues following the electrical pulse but transmission electron microscopy was not carried out to confirm this phenomenon on a smaller scale. An interesting aspect of this procedure is the very simple wiring system, allowing recording of an EPG trace, and shortly after the immobilization of the stylets for microscopy. It should be easier to use than the standard stylectomy general-

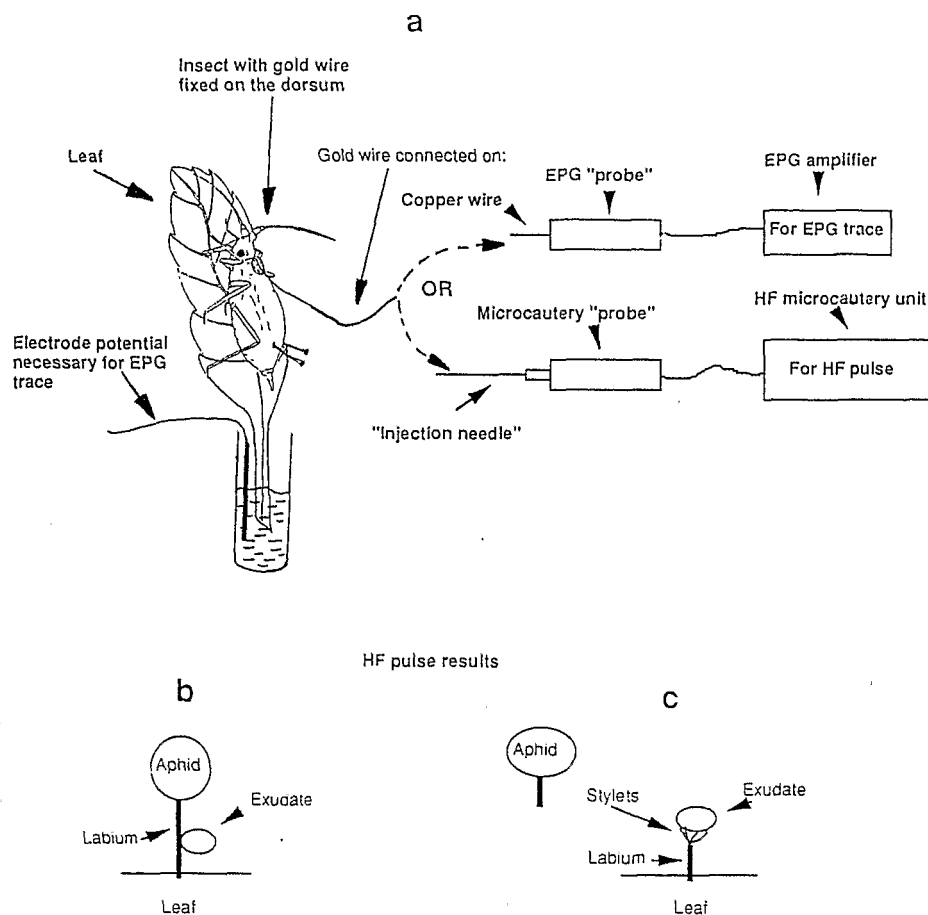


Figure 1. Schematic setup used for EPG and subsequent HF microcautery pulse. Insect wiring (a) and HF pulse results with *Macrosiphum albifrons* feeding on lupine: partial stylet amputation (b) or total stylet amputation (c).

ly employed for EPG/histology correlation (Tjallingii & Hogen Esch, 1993), especially for insects where stylectomy is extremely difficult due to their anatomy.

Interestingly and surprisingly, this technique allowed the exudation of phloem sap through the labium/stylet stump of the aphid (Figures 1b and 1c): 26 'shots' were performed on *M. albifrons* adults feeding on lupine leaves, resulting in a success rate of exudation of 69%, which is a better result than for standard stylectomy (i.e. 44% (Rahbé *et al.*, 1990)). The mean sap volume collected was 91 nl, less than in the standard technique (121 nl on leaves), with a mean flow rate of  $7 \text{ nl min}^{-1}$ . Analysis of sugars and amino acids showed that the exudate obtained was pure phloem sap (mean values: 1890 mM of sucrose and 338 mM of amino acids; no fructose/glucose contamination detected, and high amide content, both reflecting the authenticity of phloem sap sampling). According to standard analysis

for lupine (Pate *et al.*, 1979), asparagine was the main N-transporter (50%, Figure 2). The continuous exudation of phloem sap obtained after electrical shock confirmed the maintenance of the phloem's integrity, as observed by light microscopy. Nevertheless, precautions should be taken: the stylet amputation shown in Figure 1b was interpreted as a partial disruption of the mouth parts of the insect. Analyses of sugars revealed trehalose traces in such exudates, indicating contamination by haemolymph. In contrast, stylet amputations as shown in Figure 1c, resulted in uncontaminated samples (no trehalose detected). Usually, phloem exudation occurred as presented in Figures 1b and 1c. Nevertheless, we observed two cases where only the stylets were amputated, and where the labium was severed but still connected to the rest of the dead aphid. In these cases, as in standard stylectomy, the HF pulse resulted in the ejection of the aphid, leaving only the

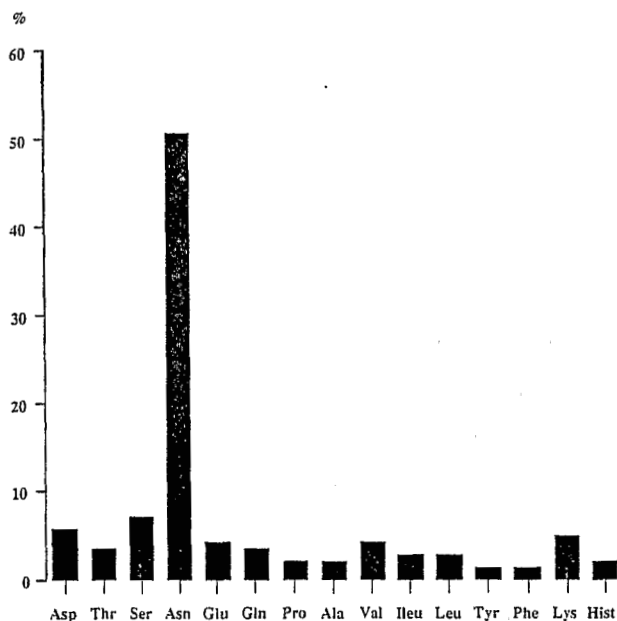


Figure 2. Typical amino acid profile of exudates obtained after HF pulse on lupine's aphid.

stylet stump, and no contamination with haemolymph occurred. These results led us to adapt the HF cautery device for better stylet amputations by changing HF frequency and power.

In contrast to our results with *M. albifrons*, styletomy was never observed with our target insect *Phenacoccus manihoti*. Whereas immobilization occurred invariably, stylet amputation seemed to be dependent on the insect species, and may not have resulted only from the electrical shock. In *M. albifrons*, stylet amputation took place relatively close to the leaf surface, and the insect twitched, excreting at the same time honeydew. Therefore, a physical reaction coupled with the HF pulse, involving some kind of vio-

lent reflex movement of the aphid head, may result in styletomy. It is surprising that phloem flow was not prevented, indicating that no overheating and no withdrawal movement occurred. The observed species-specific differences in response may be traced to differences in anatomy and behavioural reactions of the insects to the shock.

### Acknowledgments

We thank B. Boher for his help with the histological techniques used for the mealybug, and J. Guillaud and P. Jourand for their technical assistance. S. Manguin is thanked for her English corrections.

### References

- McLean, D. L. & M. G. Kinsey, 1967. Probing behaviour of pea aphid. I. *Annals of the Entomological Society of America* 60: 400-406.
- Pate, J. S., C. A. Atkins, D. L. Mc Neil & D. B. Layzell, 1979. Transport of organic solutes in phloem and xylem of a nodulated Legume. *Plant Physiology* 63: 1082-1088.
- Rahbé, Y., B. Delobel, P. A. Calatayud & G. Febvay, 1990. Phloem sap composition of lupine by aphid styletomy: Methodology, variation in major constituents and detection of minor solutes. In: D. C. Peters, J. A. Webster & C. S. Chlouber (eds), *Proceedings of Aphid-Plant Interactions: Populations to Molecules*. Oklahoma State University, Stillwater, Oklahoma, p 307.
- Tjallingii, W. F., 1978. Electronic recording of penetration behaviour by aphids. *Entomologia Experimentalis et Applicata* 24: 721-730.
- Tjallingii, W. F. & Th. Hogen Esch, 1993. Fine structure of aphid stylet routes in plant tissues in correlation with EPG signals. *Physiological Entomology* 18: 317-328.

# Entomologia Experimentalis et Applicata

Volume 80, No. 1  
July 1996

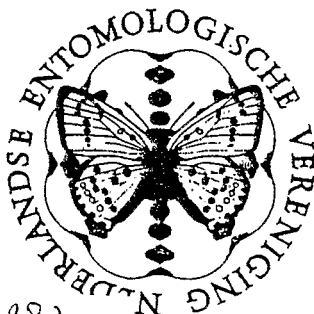
ISSN 0013-8703  
CODEN ETEAAT

## *Executive Editors*

**S.B.J. Menken** (The Netherlands)  
**A.K. Minks** (The Netherlands)  
**L.M. Schoonhoven** (The Netherlands)

## *Editors*

**E.A. Bernays** (USA)  
**S. Duffey** (USA)  
**P.B. Edwards** (Australia)  
**S. Finch** (UK)  
**A. Girardie** (France)  
**P. Guerin** (Switzerland)  
**I. Hodek** (Czech Rep.)  
**T.G.T. Jaenson** (Sweden)  
**G.G. Kennedy** (USA)  
**C. Löfstedt** (Sweden)  
**J.J.A. van Loon** (The Netherlands)  
**M. Mackauer** (Canada)  
**J.N. McNeil** (Canada)  
**J.P. Nyrop** (USA)  
**M. Rowell-Rahier** (Switzerland)  
**A. Szentesi** (Hungary)  
**S.N. Thompson** (USA)  
**L.E.M. Vet** (The Netherlands)  
**R.A. Vickers** (Australia)  
**E. Wajnberg** (France)  
**S.C. Welter** (USA)



PM 283  
5.2.7

Published for the Nederlandse Entomologische Vereniging  
by Kluwer Academic Publishers, Dordrecht (The Netherlands)