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Electropenetrography, a tool to investigate the feeding behaviour of sucking insects: development of this technique to *Scaphoideus titanus*

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Abstract: The leafhopper *Scaphoideus titanus* is the vector of the Flavescence dorée and transmits the phytoplasma causing the disease while feeding. To better understand the transmission process, we developed for this leafhopper the electropenetrograpy technique which allows studying the feeding behaviour. We used the direct current method (DC system) to study the fundamental processes of the *S. titanus* feeding behaviour. We present the first results of the characterization of the electrical patterns obtained with this insect.

Key words: *Scaphoideus titanus*, grapevine, EPG, probing behaviour

Introduction

Studying the feeding behaviour patterns in the insect vectors is of primary importance because it conditions the disease transmission. In piercing-sucking insects feeding patterns can hardly be observed. Two techniques can either be coupled or not: the elegant technique of Electropenetrography (EPG) and the microscopy reconstruction of the stylets pathways inside the plant tissues. The EPG technique has been popularized by Tjallingi's in the 80's and by further works, especially on aphids. By connecting the insect and the plant in an electrical circuit it is possible to monitor electrical resistance fluctuations during probing. These voltage fluctuations occur in a number of distinctive patterns called waveforms that can be correlated to different behaviors according to the stylets position into the leaf and to the feeding activity (salivation, puncture, ingestion, etc…).

The causative agent of the FD, "*Candidatus Phytoplasma vitis*" is transmitted from one grapevine to another by the leafhopper *Scaphoideus titanus* (Homoptera: Cicadellidae). The acquisition of the pathogenic from one infected grapevine occurs during phloem ingestion, and phytoplasma innoculation to healthy grapevine by the vector takes place with salivation. These two steps of the FD biological cycle are decisive and should be better known in order to develop new control methods. For that reason, we have adapted the EPG technique to *S. titanus* in order to improve our knowledge of its feeding behaviour, and to better understand the events in insect vector-plant interactions that could be involved in the disease transmission. We thus assume that feeding behaviour of this insect can be related to some extent to the epidemiology of the FD.

Material and methods

Tested insects and plants

Scaphoideus titanus larvae were obtained as described in Caudwell *et al.* (1970). Egg hatchings were obtained by placing two year-old woody canes inside plastic hatching cages in a climatic chamber under a 16:8 (L:D) photoperiod, at 23 ± 1°C, and 65–70% RH. In order to collect neonate larvae, six cutting leaves of Cabernet-Sauvignon cultivar, maintained in a glass tube with water, were added in each cage about 20 days after the eggs were removed from the climatic chamber. Leaves were replaced when they began to wither.

Grapevine cuttings cv Cabernet-Sauvignon were produced in our greenhouse. The cuttings were cultivated in indivual pots. We used 8/9 leaves plants, above 30cm, at the same age.

Probing behaviour recording

We recorded the probing behavior of *S. titanus* adults (males and females) by using a DC-EPG system (Giga-8; EPG system, Wageningen, the Netherlands).

Leafhoppers were stored individually in Petri dishes (Ø 8.5cm) at 4°C for 15 min to reduce their activity. Then, Petri dishes containing the leafhoppers were placed on ice until the beginning of the trial. A segment of gold wire (Ø 18.5µm), around 5cm long, was glued on one extremity on the prothorax of the insect with silver glue (EPG system, Wageningen, the Netherlands), and on the other end to the insect electrode (copper pin) with the same glue (Figure 1). After the leafhopper was connected, the insect electrode was inserted into EPG probe. Then, the plant electrode (copper, 5cm long, Ø 2mm) was inserted into the soil plant. The electrical signals from the feeding behaviour of the insect were amplified and converted from analogic to numeric signal. Signals were recorded on a PC with the software Probe 3.5. The probing behavior was recorded simultaneously on 4 cuttings with only one adult on each plant for 4 h. Fifteen individuals were recorded.

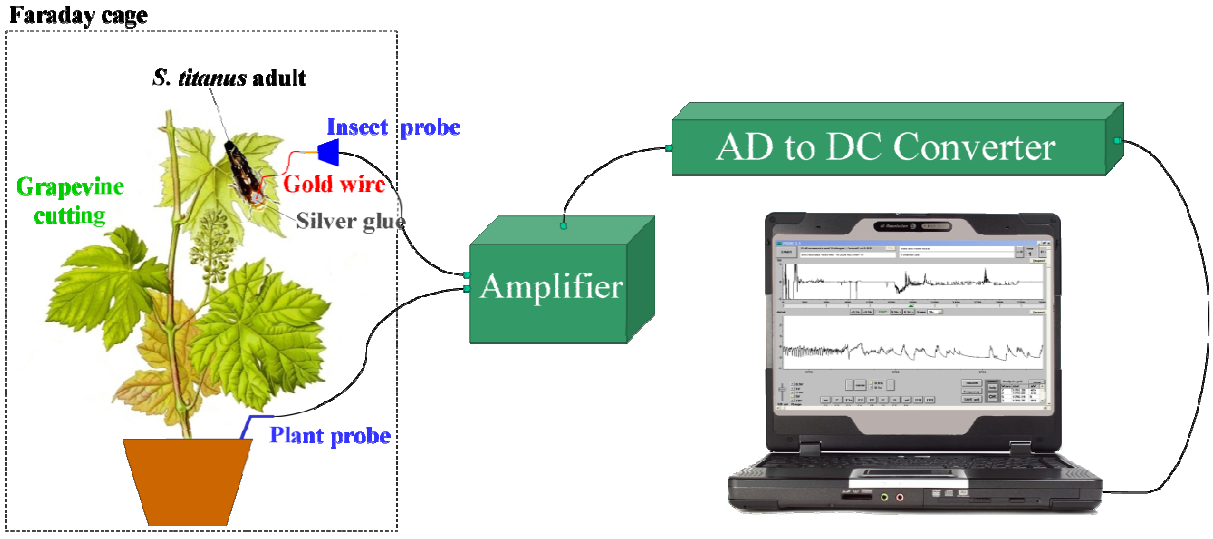

Figure 1. Experimental design. Details are provided in the text.

Recording analysis

Recordings were analysed with the Probe 3.5 software. Electrical patterns were identified as characteristics when observed either on different insects or/and several times in the same

individual. Later on, the "characteristic patterns" have been compared to those mentioned in the litterature and to the general knowledge about the behaviour of piercing-sucking insects.

Results and discussion

Potential drop

As shown in Figure 2, we recorded potential drops that, as far as we know, are very uncommon in leafhoppers. Indeed, if potential drops are present in in most recordings of Sternorrhyncha (i.e., aphids, whiteflies, and mealybugs), they are not present or rare in Auchenorrhyncha (i.e., cicadas, leafhoppers, treehoppers, planthoppers, and spittlebugs). (Miranda *et al.*, 2009). In aphids, potential drops occur when their stylet tips puncture through the plasmalemma of living plant cells (Tjallingii, 1985) and phloem ingestion begins after a potential drop. In our recording, none phloem ingestion waveform follows a potential drop.

Figure 2. Electrical penetration graph of *S. titanus* on grapevine showing a potential drop. Abscissa is in seconds, ordinate is in mV.

Salivation

We have recorded some pattern linked to the activity running of the cibarial pump muscles (Figure 3) which are involved in the active xylem ingestion. Their characterization was made with the correlation between EPG waveforms and electromyographically recording from the cibarial dilator muscles (Dugravot *et al.*, 2008).

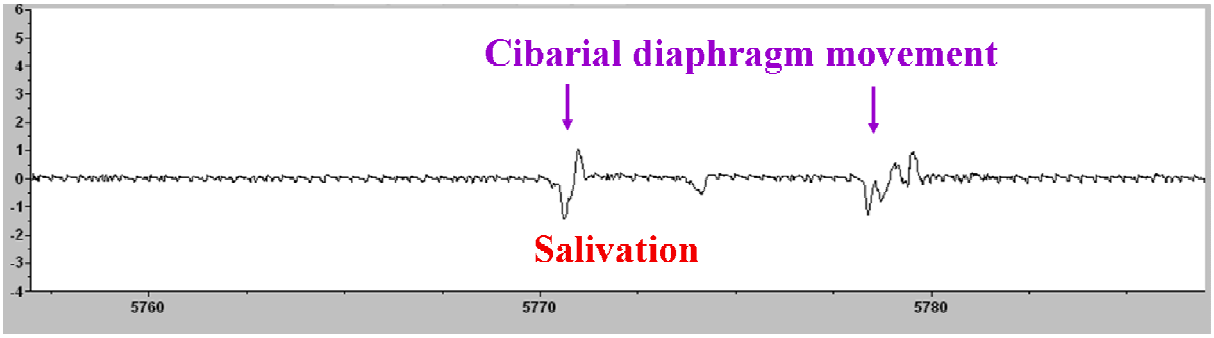

Figure 3. Electrical penetration graph of *S. titanus* on grapevine showing cibarial diaphragm movement due to the activity of the cibarial dilator muscles. Abscissa is in seconds, ordinate is in mV.

Xylem ingestion

Xylem feeding pattern is characterized by waveforms with high amplitude occurring at a very constant repetition rate with few shape variations (Figure 4). This type of signal has already been correlated to xylem sap ingestion like in the beet leafhopper *Circulifer tenellus* (Stafford and Walker, 2009).

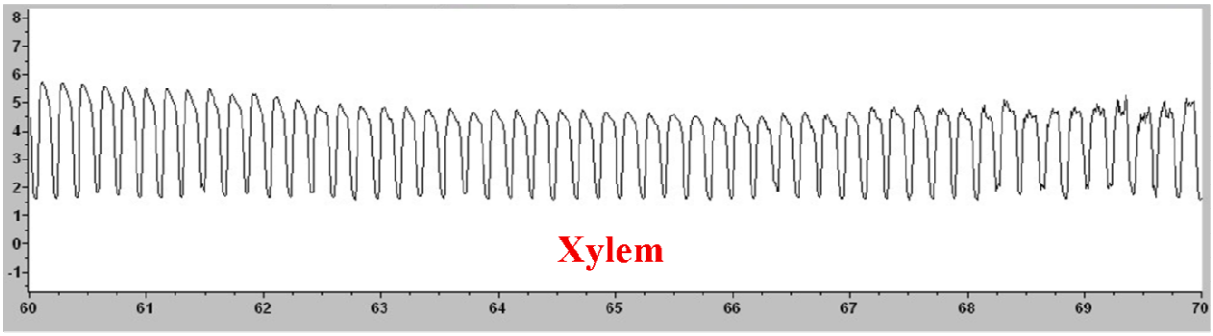

Figure 4. Xylem waveform. Abscissa is in seconds, ordinate is in mV.

Phloem ingestion

Phloem ingestion produced a characteristic waveform beginning with a vertical rise of voltage level and ending with a quick drop (Figure 5) like in the planthopper *Perkinsiella saccharicida* (Chang, 1978). All observed phloem ingestion pattern showed two different phases. After the first high amplitude phase, a second one with lower amplitude and higher frequency begins after a brief increase of the voltage level. These two successive phases could be related with the two phases of the phloem ingestion in aphids (Prado and Tjallingii, 1994)

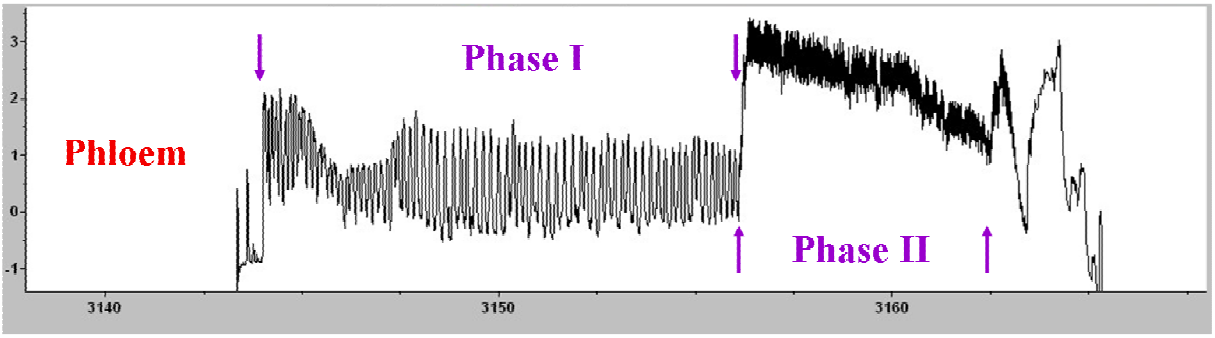

Figure 5. Phloem waveform. Abscissa is in seconds, ordinate is in mV.

The characterization of the observed waveforms is in progress and will lead to a fine exploration of the feeding behaviour of *S. titanus*. The occurrence and the duration of the different waveforms could be related to the phytoplasma transmission phases and could lead to a better understanding of Flavescence dorée transmission and of differential cultivar susceptibility. Indeed, feeding behavior influences some decisive parameters at the phytoplasma/vector relationships (e.g. acquisition and inoculation rate) and is thus important to understand the disease epidemiology.

This work represents a first step in the understandig of the physiological process of the Flavescence dorée phytoplasma transmission to grapevine by *S. titanus*.

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