Integrated protection and production in Viticulture
IOBC-WPRS Bulletin Vol. 105, 2014
pp. 177-183

Can differences in feeding behaviour between *Scaphoideus titanus* males and females be related to phytoplasma transmission efficiency?

J. Chuche[1,2], D. Thiéry[1,2]
[1]*UMR Santé et Agroécologie du VignoblE (SAVE), INRA, 33883 Villenave d'Ornon, France;*
[2]*UMR Santé et Agroécologie du VignoblE (SAVE), Bordeaux Sciences Agro, 33883 Villenave d'Ornon, France*
E-mail: jchuche@bordeaux.inra.fr; thiery@bordeaux.inra.fr

Abstract: The leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae) is a vector of the phytoplasma causing the Flavescence dorée of grapevine which is one of the most economically threatening disease of European vineyards. In plant disease solely transmitted by insects, pathogen transmission occurs during the feeding behaviour, therefore quantifying the different feeding activities is a key to understand the disease transmission process. Feeding behaviour of piercing-sucking insects can be studied using the electrical penetration graph technique (EPG). This technique has been popularized by Tjallingi in the 1980s and 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 have been correlated to different behaviours according to the stylets position into the leaf and to feeding activity (salivation, puncture, ingestion, etc…). We have adapted this technique to *S. titanus* in order to investigate if differences in the feeding behaviour between non infected males and females could explain different ability in phytoplasma transmission. EPG waveforms representing probing activities were obtained from adult *S. titanus* probing in Cabernet Sauvignon cultivar. Three waveforms: salivation, phloem and xylem ingestion were characterized in both sexes by comparing them to previously published ones for other Hemipteran species. The first interesting result is that xylem ingestion occurred in both sexes, while *S. titanu*s is always described as a phloem feeder. Interestingly, males exhibited more frequent and longer activity in phloem. The number, mean and total durations of each type of waveform differed significantly depending on the sex. Feeding behaviour differences affect the ability to acquire and then to inoculate phytoplasma and may partly explain the higher rates of transmission of Flavescence dorée phytoplasma that were obtained in the laboratory with males.

Key words: electropenetrography, Flavescence dorée, vector, phloem, xylem

Introduction

Currently, European viticulture is confronted with two serious phytoplasma diseases, Flavescence dorée (FD) and bois noir, with FD being caused by phytoplasma strains of the elm yellows group (16SrV) (Angelini *et al.*, 2003). The FD transmission is highly species-specific and the leafhopper vector *Scaphoideus titanus* is a specialist of *Vitis* in Europe (Chuche & Thiéry, 2014). The transmission of phytoplasma strains responsible for FD is species-specific in Europe, with *S. titanus* not being able to transmit other phytoplasma responsible for grapevine yellows (Carraro *et al.*, 1994). FD phytoplasma has a high degree of specificity to its vector, and movements made by the insect from one plant to another constitute the only natural transmission paths. Thus, feeding behaviour of this insect is the determining parameter involved in FD transmission and spread.

Due to its status of phytoplasma vector, *S. titanus* , was up to now described as a phloem-feeder. Phytoplasma acquisition is a proof of phloem consumption, but it doesn't exclude another feeding site and we can logically ask whether *S. titanus* has not a more varied diet However, ancient microscopic observations of cut petioles (Carle & Moutous, 1965; Schvester *et al.*, 1962; Vidano, 1964) showed that many salivary sheath ended in the xylem The fact that stylets reach the xylem does not necessarily means that the insect intakes sap. It can simply taste without feeding in xylem.

Anatomical studies revealed the existence of a well differentiated filter chamber with extremely thin epithelial cells, reduced cytoplasm and abundant microvilli (LeCaherec *et al.*, 1997). The existence of such anatomical structures is typical of xylem-feeder. Moreover, immunogold labelling with anti-aquaporin antiserum on *S. titanus* filter-chamber ultrathin sections showed the presence of aquaporin-like proteins that allow water transfer through the filter chamber (LeCaherec *et al.*, 1997). That's also characteristic of xylem sap feeders. Thus, *S. titanus* is a phloem-feeder having typical xylem-feeder structures.

As it was reported in numerous leafhopper vector species, males and females can have different ability to transmit pathogens. Regarding *S. titanus*, studies conducted in the US on American grapevine yellows (Maixner *et al.*, 1993) and in Europe on Flavescence dorée (Lessio *et al.*, 2009; Schvester *et al.*, 1969) showed that the proportion of males carrying phytoplasma is higher than that of females. So, in a vineyard with a FD inoculum, more males will be carriers of the phytoplasma and potentially they will contribute more to FD transmission. In transmission trials, after phytoplasma acquisition on diseased grapevines, males transmit FD with a higher efficiency than females. To resume, males are better vectors than females because more individual carry phytoplasma in vineyard and transmit the disease with a higher efficiency.

In this work, we adapted the electropenetrography technique (EPG) tool to this leafhopper species in order to discriminate behavioural differences related to phytoplasma transmission. To do this, we correlated the profiles obtained in EPG feeding activity of *S. titanus* by comparing them with data available in the literature. We also used EPG to determine if *S. titanus* feed only on phloem and if males and females have similar feeding patterns.

Material and methods

Biological materials

Male and female imagos were obtained from wild populations as previously described (Chuche & Thiéry, 2009). Two-year-old grapevine woody canes carrying eggs were collected in an organic vineyard in Burgundy where important populations of *S. titanus* occurred for successive years. Egg hatching was obtained by placing wood pieces bearing eggs inside plastic hatching cages in a climatic chamber under a 16:8 (L:D) photoperiod, at 23 ± 1 °C, and 65-70% R.H. In order to harvest neonate larvae, six leaves taken from healthy cuttings of Cabernet-Sauvignon cultivar were maintained in a glass tube containing water and placed into the cage. Every day, larvae were gently removed from the lower side of leaves with a pooter and placed into cages with healthy Cabernet-Sauvignon cuttings until they reached the adult stage.

EPG records

EPG allow to record resistance variations that are related to feeding patterns of sucking insect. These variations produce different waveforms corresponding to specific behaviours reflecting

the position of the stylet in plant tissue and feeding activity. The experimental device used is equivalent to that developed by Tjallingii (1985). The recordings were made with a DC EPG (Giga-8; EPG system, Wageningen, The Netherlands). Insects, plants, and the electrodes were isolated from background noise by a Faraday cage. The electrical signals picked up by the electrodes were amplified and converted into digital signals via the acquisition system Di710-UL (DATAQ, Akron, USA), then visualized and recorded on a computer using Probe 3.5 software (EPG system, Wageningen , Netherlands). The entire device was placed in an air-conditioned room to keep a constant temperature (23 ± 1 °C).

Leafhoppers were taken from their breeding cage and then placed individually in Petri dishes (diameter 8.5 cm) and stored at 4 °C for 15 min in order to facilitate handling. Petri dishes were then placed on a bed of ice until their use. In order to fix the gold wire on adults, the insect was held stationary at the end of a plastic tip with a slight suction. An approximately 5 cm gold wire (Ø 18.5 µm) was connected to the end of the pronotum with a drop of silver glue (EPG system, Wageningen, The Netherlands) under a dissecting microscope (M 7, Wild Heerbrugg, Gays, Switzerland). Once the insect was attached, the other end of the gold wire was connected to the copper end of an electrode also using silver glue. The electrode was then inserted into the EPG probe and the insect deposited on the plant. Finally, a second copper electrode (5 cm, diameter 2 mm) was put into the substrate of the cutting.

Records were carried out simultaneously on four cuttings, each hosting one adult, for a period of 4 h. Visual observation of the insects behaviour was carried out simultaneously in order to better correlate the signals observed with insect behaviour. Fifteen individuals of both sexes were used for the characterization of signals and 30 males and 30 females were used for experimentation comparison of the two sexes.

Signal analysis

Signals were analyzed using the software Probe 3.5. Characteristic signals retained were observed for several insects and several times for the same individual. These signals were then compared to those described in the literature and general knowledge about the behaviour of sucking insects. Selected signals are those corresponding to the active xylem ingestion, passive ingestion of phloem and salivation. The criteria used to compare the feeding behaviour of males and females in their relationship to pathogen transmission and acquisition were the number of signals corresponding to the xylem, phloem and salivation, their average duration and total duration.

The parameters considered for comparing feeding behaviour of males and females were analyzed using a Mann Whitney test. Statistical analyses were performed using the software R 2.8.0 for Windows (R Development Core Team, 2007).

Results and discussion

Signal characterization

A typical profile corresponding to xylem sap feeding is shown in Figure 1. This kind of waveform is characterized by large amplitude signals, generally greater than 0.5 mV and frequently above 1 mV. This very regular signal with large amplitude is similar to those observed in other leafhopper vectors as *Cicadulina mbila* on corn (Lett *et al.*, 2001) and *Circulifer tenellus* on sugar beet (Stafford & Walker, 2009).

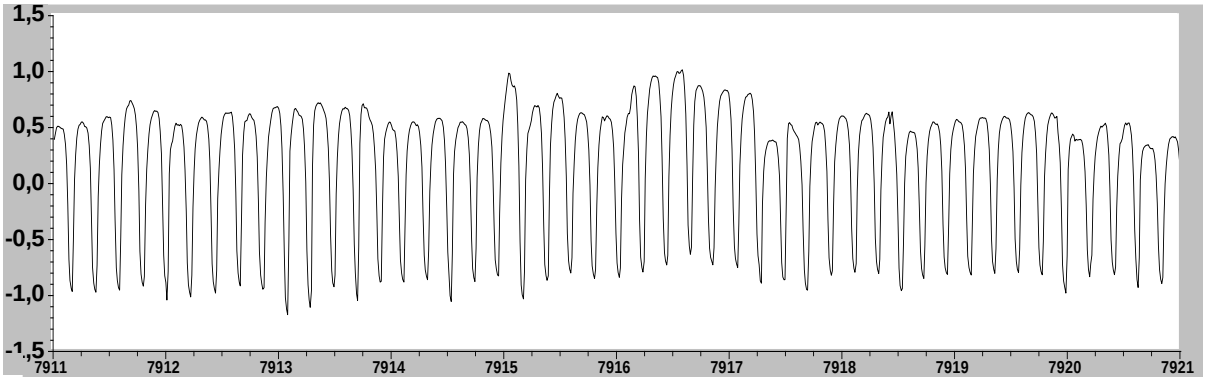

Figure 1. Electrical penetration graph waveforms produced by *S. titanus* corresponding to xylem sap ingestion. Time (s) is plotted on the abscissa whereas electrical variation (V) is plotted on the ordinate.

Figure 2 illustrates a record corresponding to phloem sap ingestion by *S. titanus*. Waveforms produced by phloem sap ingestion present varying profiles but have in common low average amplitudes, less than 25 mV, a fairly regular succession of the same signal shape, but less than xylem signals. Shape variation of the observed signals can be partly explained by the different activities inside the phloem, as well as the mode of phloem ingestion. Signals obtained corresponded to those described in *C. mbila* feeding on maize (Lett *et al.*, 2001) and *Empoasca vitis* on tea (Jin & Baoyu, 2007).

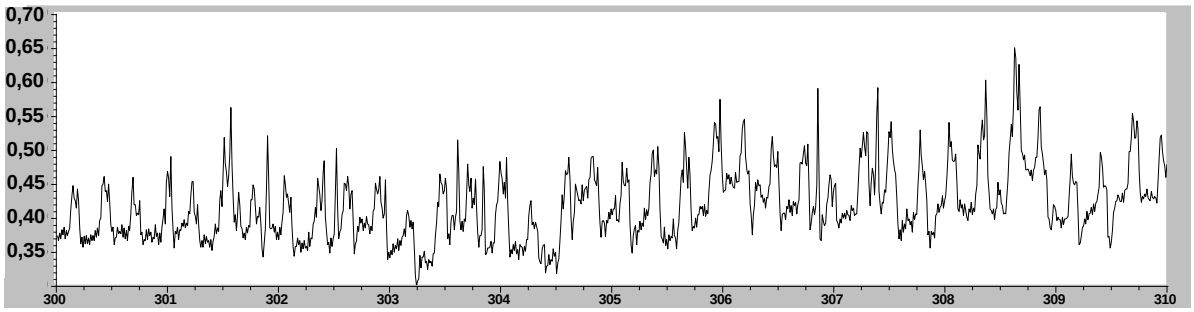

Figure 2. Electrical penetration graph waveforms produced by *S. titanus* corresponding to phloem sap ingestion. Time (s) is plotted on the abscissa whereas electrical variation (V) is plotted on the ordinate.

Signals corresponding to phloem and xylem had a moderate frequency, about 5 Hz, and can be interrupted by a profile of low amplitude with high frequency, about 20 Hz, and preceded by a rapid increase of the voltage as shown by the Figure 3. These signals corresponding to salivation were also observed in the sharpshooter *Homalodisca coagulata* feeding on grapevine (Backus *et al.*, 2005) and in *C. tenellus* feeding on beetroot phloem (Stafford & Walker, 2009).

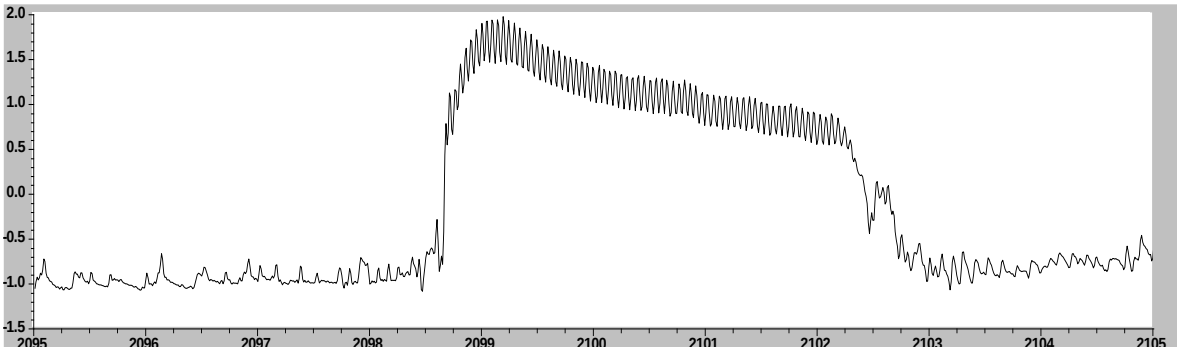

Figure 3. Electrical penetration graph waveforms produced by *S. titanus* corresponding to salivation. Time (s) is plotted on the abscissa whereas electrical variation (V) is plotted on the ordinate.

Male and female feeding behaviour

Our results confirm salivary sheath observation by showing that *S. titanus* probed in xylem. About 85 % of males (25/30) and more than 95 % of females (29/30) probed at least once in xylem. Interestingly, females probed less in phloem (16/30) than males (26/30). Salivation occurred in the same proportions for males (21/30) and females (23/30).

Figure 4 represents the mean number of probing in phloem and xylem by the two genders. There is no difference between xylem and phloem for males, but females probed more in xylem than phloem. In the same way, females probed longer in xylem than in phloem. Thus, *S. titanus* has a mix diet and feed equally in phloem and xylem for males, and more on xylem for females.

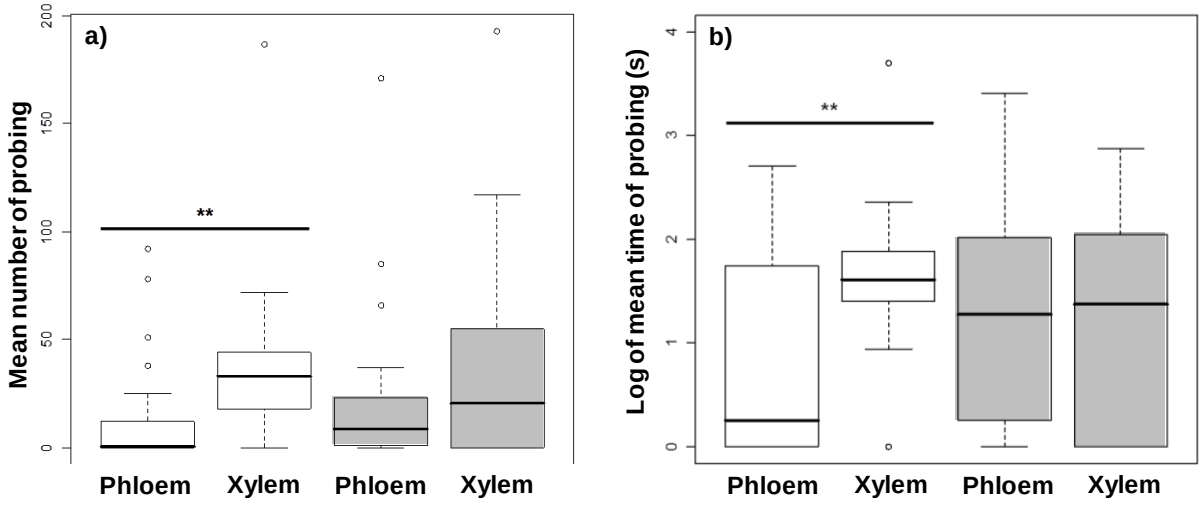

Figure 4. Sap intake by *S. titanus*: a) mean number of sap probing for females (open boxes) and males (shaded boxes); b) mean time of sap probing for females (open boxes) and males (shaded boxes). ** $p < 0.01$, Mann Whitney test.

Figure 5 shows the mean number of phloem probing, salivation and xylem probing for females and males. There is no difference between the two genders for salivation and xylem, but females probed less frequently than males in phloem. In the same way, males probed longer in phloem than females.

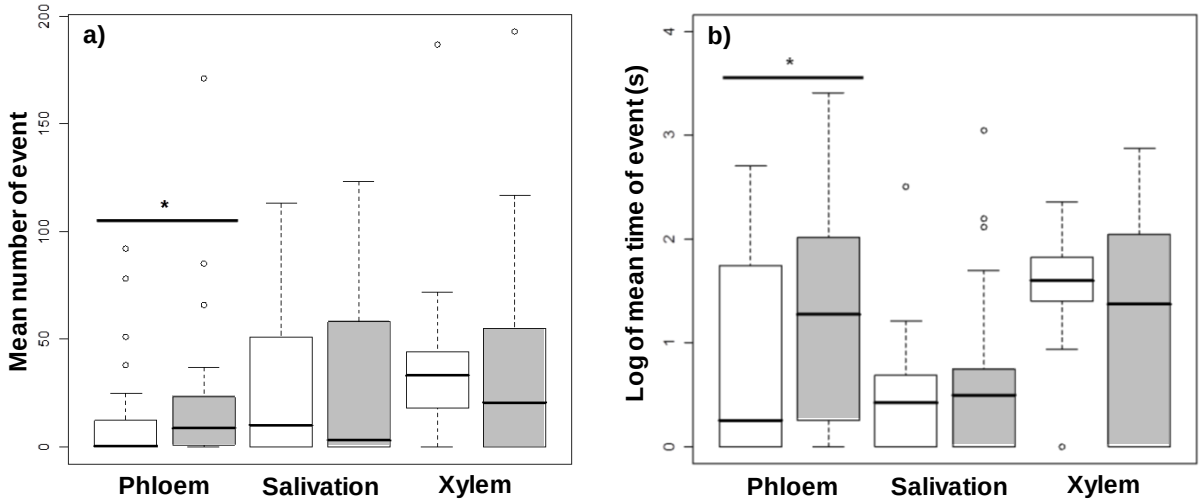

Figure 5. Feeding behaviour of males and females *S. titanus*: a) mean number of behavioural event for females (open boxes) and males (shaded boxes); b) mean time for each behavioural event for females (open boxes) and males (shaded boxes). * $p < 0.05$, Mann Whitney test.

Because males probe more frequently and last longer in the phloem than females, they have a highest probability to acquire phytoplasma and, once infective, to inoculate it. This feeding behaviour makes males better vectors than females and could partly explain differences in transmissions efficiency observed. To conclude, S. titanus is not only a phloem-feeder but also feed on xylem. Therefore, the different feeding behaviour can partly explain that males are better vectors than females. This mixed feeding behaviour is uncommon and lead to questioning about the interest to feed both on xylem and phloem.

Acknowledgements

This work is part of the Labex COTE project and has been supported by grants from the Conseil Interprofessionnel des Vins de Bordeaux, the Aquitaine region and INRA Santé des Plantes et Environnement department.

References

Angelini, E., Negrisolo, E., Clair, D., Borgo, M., Boudon-Padieu, E. 2003: Phylogenetic relationships among Flavescence dorée strains and related phytoplasmas determined by heteroduplex mobility assay and sequence of ribosomal and nonribosomal DNA. Plant Pathol. 52: 663-672.

Backus, E. A., Habibi, J., Yan, F. M., Ellersieck, M. 2005: Stylet penetration by adult *Homalodisca coagulata* on grape: Electrical penetration graph waveform characterization, tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. Ann. Entomol. Soc. Am. 98: 787-813.

Carle, P., Moutous, G. 1965: Observations sur le mode de nutrition sur vigne de quatre espèces de cicadelles. Ann. Epiphyt. 16: 333-354.

Carraro, L., Loi, N., Kuszala, C., Clair, D., Boudon-Padieu, E., Refatti, E. 1994: On the ability-inability of *Scaphoideus titanus* to transmit different grapevine yellow agents. Vitis 33: 231-234.

Chuche, J., Thiéry, D. 2014: Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*. A review. Agron. Sustain. Dev. in press.

Chuche, J., Thiéry, D. 2009: Cold winter temperatures condition the egg-hatching dynamics of a grape disease vector. Naturwissenschaften 96: 827-834.

Jin, M., Baoyu, H. 2007: Probing behavior of the tea green leafhopper on different tea plant cultivars. Acta Ecol. Sin. 27: 3973-3982.

LeCaherec, F., Guillam, M. T., Beuron, F., Cavalier, A., Thomas, D., Gouranton, J., Hubert, J. F. 1997: Aquaporin-related proteins in the filter chamber of homopteran insects. Cell Tissue Res. 290: 143-151.

Lessio, F., Tedeschi, R., Pajoro, M., Alma, A. 2009: Seasonal progression of sex ratio and phytoplasma infection in *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bull. Entomol. Res. 99: 377-383.

Lett, J.-M., Granier, M., Grondin, M., Turpin, P., Molinaro, F., Chiroleu, F., Peterschmitt, M., Reynaud, B. 2001: Electrical penetration graphs from *Cicadulina mbila* on maize, the fine structure of its stylet pathways and consequences for virus transmission efficiency. Entomol. Exp. Appl. 101: 93-109.

Maixner, M., Pearson, R. C., Boudon-Padieu, E., Caudwell, A. 1993: *Scaphoideus titanus*, a possible vector of Grapevine Yellows in New York. Plant Dis. 77: 408-413.

Schvester, D., Carle, A., Moutous, G. 1969: Nouvelles données sur la transmission de la Flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. Ann. Zool. Ecol. Anim. 1: 445-465.

Schvester, D., Moutous, G., Bonfils, J., Carle, P. 1962: Étude biologique des cicadelles de la vigne dans le Sud-Ouest de la France. Ann. Epiphyt. 13: 205-237.

Stafford, C. A., Walker, G. P. 2009: Characterization and correlation of DC electrical penetration graph waveforms with feeding behavior of beet leafhopper, *Circulifer tenellus*. Entomol. Exp. Appl. 130: 113-129.

Tjallingii, W. F. 1985: Electrical nature of recorded signals during stylet penetration by aphids. Entomol. Exp. Appl. 38: 177-186.

Vidano, C. 1964: Scoperta in Italia dello *Scaphoideus littoralis* Ball cicalina americana collegata alla «Flavescence dorée» della Vite. Ital Agric. 101: 1031-1049.