INVESTIGATING VECTOR SPECIFICITY OF FLAVESCENCE DORÉE PHYTOPLASMA OF SOME HEMIPTERA SPECIES

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Introduction

Identification of vectors and potential vectors of Grapevine yellows (GY) diseases is crucial for epidemiology and control of these important diseases (Boudon-Padieu, 2005). Few information are reported in literature concerning the specificity of vector transmission of phytoplasmas. Some phytoplasma strains seem to be specifically transmitted, for example phytoplasmas in the Elm yellow group seem to be transmitted by only a few leafhopper species. Other strains have low vector specificity, such as those clustered in the Aster yellows group, which are transmitted by several leafhopper species. Flavescence dorée (FD) of the grapevine is associated with a phytoplasma (FDP) that seems to be specifically transmitted in the field by the leafhopper vector *Scaphoideus titanus* Ball (Hemiptera, Cicadomorpha, Cicadellidae) (Schvester *et al*., 1963; Vidano, 1964; Mori *et al*., 2002). However Caudwell *et al*. (1972) showed that in experimental conditions, *Euscelidius variegatus* Kirschbaum (Hemiptera, Cicadomorpha, Cicadellidae) was an efficient alternative vector of FDP to herbaceous hosts although it cannot feed-acquire FDP from infected grapevines. Hence the species of source and recipient plants are very important both in natural diffusion and experimental assays.

In the present work we studied the vector specificity of FDP by 15 Hemiptera Cicadomorpha and Fulgoromorpha species collected in European vineyards. To test vector transmission we adopted needle injection to deliver phytoplasma suspensions into the abdomen of candidate vector species (Purcell, 1996). After an incubation period we fed the injected insects on artificial diets through a Parafilm membrane (Zhang *et al*. 1998; Tanne *et al*. 2001) and tested the feeding medium for the presence of FDP DNA using PCR amplification of FDP DNA.

Materials and Methods

Healthy colonies of *E. variegatus* were maintained on maize inside cubical Plexiglas cages and used for feeding acquisition and transmission on broadbean to maintain phytoplasma strains (Caudwell and Larrue, 1977). Healthy nymphs or young adults were periodically collected for injection assays from the latter colonies.

Suspensions of FDP were obtained from FDP-infective *E. variegatus* according to Whitcomb and Coan (1982). Optimization of the concentration of viable phytoplasma extracts and latency in vectors were monitored by injecting healthy-reared *E. variegatus* leafhoppers. Then insects were injected using phytoplasma extracts that ensured the highest rate of FDP acquisition and transmission by *E. variegatus.* Transmission was attempted to an artificial diet through a Parafilm membrane about three weeks after insect injection.

Leafhopper and planthopper specimens were collected by using a D-vac or a sweep-net in viticulture areas located in the Mosel Region(D), Burgundy Region (F) and Veneto Region (I) or kindly provided by other laboratories. Insects were caged on suitable host plants. Trapped univoltine species were directly used for injection assays, while plurivoltine species were reared for at least one generation and the progeny was used in injection assays. All the insect species were maintained or reared in a climatic chamber (23 ± 1 ºC, L16:D8).

To confirm transmissibility of FDP by some tested species, insects were confined on FD-infected broadbeans or on healthy broadbean seedlings (as control) for an acquisition period of 15 days and then confined to healthy broadbean seedlings after an incubation period of about 35 days.

Detection of FDP in insects, in plants and in feeding medium (Zhang *et al*. 1998; Tanne *et al*. 2001) was done with PCR amplification of phytoplasma DNA using FDP-specific primers (Clair *et al*. 2003).

Results

Among the batches of FDP-injected insects belonging to 15 Hemiptera species, that were confined in cages and fed through a Parafilm membrane in the medium for a 4-5 days inoculation access period (IAP), FDP DNA was detected by PCR in the feeding medium inoculated by the leafhoppers (Cicadellidae) *Anoplotettix* fuscovenosus (Ferrari), *Aphrodes makarovi* Zachvatkin, *Euscelidius variegatus* Kirschbaum and *Euscelis incisus* Kirschbaum but not in the feeding medium where injected insects of the other 11 species were confined: the leafhoppers *Agallia consobrina* Curtis *Circulifer haematoceps* (Mulsant & Rey), *Fieberiella florii* (Stål), *Psammotettix* sp.; the spittlebug (Cercopidae) *Philaenus spumarius* (Linneus); the treehopper (Membracidae) *Stictocephala bisonia* (Kopp &Yonke); the Fulgoromorpha: *Agalmatium flavescens* (Olivier), *Hyalesthes obsoletus* Signoret, *Laodelphax striatellus* (Fallén), *Metcalfa pruinosa* (Say), and *Pentastiridius* sp.. Detection of FDP was positive in injected insects of all the Hemiptera species although band intensities in the agarose gels were positively associated with the transmissibility of FDP to artificial diets.

The ability of *E. variegatus* and *E. incisus* and the inability of *C. haematoceps* and *F. florii*, to transmit FDP, were confirmed by feeding the insects for acquisition on FDP-infected broadbeans then for transmission on healthy broadbean seedlings. FDP in inoculated broadbeans was confirmed by symptom expression and by PCR detection. *E. variegatus* and *E. incisus* that transmitted to feeding medium also inoculated broadbean; *C. haematoceps and F. florii* transmitted neither to feeding medium nor to broadbean.

Discussion

Injection technique is a potential useful tool for searching for insect vectors (Whitcomb and Coan, 1982) because it increases the rate of phytoplasma acquisition and reduces the latent period. Additionally it is possible to test insects with different feeding habits or different host plant preferences.

The use of feeding medium assays to test the inoculative potential of insects allows a significant reduction of time if compared with transmission to host plants. Also, it eliminates the effects of host plant-vector interactions in the inoculation process. In addition, Ge and Maixner (2003) found that the technique increased the efficiency of vector transmission when compared to feeding transmission to host plants.

The four insect species that transmitted FDP after injection belong to the family Cicadellidae, as well as the economic vector of FDP, *S. titanus*. The other Hemiptera species tested could not transmit FDP after abdominal injection of the phytoplasma suspension. Therefore we may assume that the latter species are not potential vectors of FDP or are extremely inefficient in transmitting the mollicute. Passage of plant pathogens from the haemocoel to the salivary glands and subsequent transmission is not enough by it self to recognize if one insect is a vector. Actually, in natural conditions, phytoplasma cells should overcome at least the two physical barriers that are the gut and the salivary glands (Lefol *et al*., 1994); Fletcher *et al*., 1998). Therefore other assays based on feeding acquisition from FDP-infected host plants are in progress to confirm the ability to transmit FDP by *A. fuscovenosus* and *A. makarovi*.

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