Microinjection and insect feeding medium bioassays to test the vector specificity of Flavescence dorée phytoplasma by some Hemiptera species

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Phytoplasmas are a group mollicute plant pathogens transmitted by insect vectors in the Hemiptera. Although vector species have been identified for many of plant diseases associated with phytoplasmas, little is known about the specificity of vector transmission. 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 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). However earlier works revealed that, in experimental conditions, other leafhopper species were alternative vectors of FDP to herbaceous hosts, i.e. *Euscelidius variegatus* Kirschbaum (Hemiptera, Cicadomorpha, Cicadellidae) (Caudwell et al. 1972).

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), and 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 by a PCR procedure.

Materials and Methods

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

Suspensions of FDP were obtained from FDP-infective *E. variegatus* following a procedure described by 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. On the basis of these preliminary results, 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 (feeding medium) 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 (Germany), Burgundy Region (France) and Veneto Region (Italy), other specimens were kindly provided by some specialized laboratories. Insects were caged on suitable host plants. 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 insects, we attempted transmission assays by insect feeding on plants. To accomplish this, 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 that acquired FDP by injection or by feeding, in plants and in feeding medium was done using polymerase chain reaction (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.

FDP was not detected 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/inability to transmit FDP was confirmed for *E. variegatus*, *E. incisus*, *C. haematoceps* and *F. florii* by feeding on FDP-infected broadbeans and transmission to 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). Injecting pathogens into the insect haemocoel increases the transmission efficiency by vectors by increasing the rate of phytoplasma acquisition and reducing the latent period. Additionally it is possible to test insects with different feeding habits or insects that have different host plant preferences and that could not feed well on plants used as source for acquisition. As a result injection of pathogen in the abdomen of the vectors suppresses the effects of host plant-vector interactions in the acquisition process.

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, considering the time necessary for symptom expression in inoculated plants (incubation period). Also, it eliminates the effects of host plant-vector interactions in the inoculation process. In addition, the technique allows in increasing the efficiency of vector transmission when compared to feeding transmission to host plants (Ge and Maixner 2003).

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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