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Research Note

Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae)

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Yellows diseases of grapevine are an increasing problem in several viticultural regions of the world. While the pathogen of Flavescence dorée (FD) is known to be a mycoplasma-like organism (MLO) and the spread of this yellows by a leafhopper vector is well understood, information on etiology and epidemiology of other grapevine yellows is sparse. Recently, MLOs were detected by PCR in grapevines with symptoms of Bois Noir, Vergilbungskrankheit (VK), and other yellows diseases (Daire *et al.* 1993 a, 1993 b; Maixner *et al.* 1994). Observations of spatial and temporal dynamics of yellows diseases indicate a natural spread in vineyards, but vectors have not yet been identified.

A three-year survey of the leafhopper fauna of vineyards led to the identification of more than 40 species of Auchenorrhyncha, several known to be vectors of MLOs. For transmission experiments, we focused on the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae) a common species in vineyards. This vector of the Stolbur disease of solanaceous plants (Fos *et al.* 1992) is occasionally present on grapevine leaves. Adult *H. obsoletus* were collected individually from grapevine and from *Convolvolus arvensis* (bindweed) and *Solanum nigrum*, two common weeds in vineyards. *H. obsoletus* was preferably collected from bindweed with severe stunting symptoms. Stunted specimens of bindweed as well as Solanum plants showing virescence were included in this study.

Groups of 4-15 insects were fed on grapevine seedlings for 14 days. DNA extraction from planthoppers, grapevine, and herbaceous hosts, the PCR amplification and subsequent restriction analysis were performed following the procedure of Ahrens and Seemüller (1992).

Symptoms of MLO infection developed in 4 of 10 grapevine seedlings on which *H. obsoletus* had been fed, although more than 80 % of the planthoppers died within 5 d on grapevine. The stems of these vines, which were severely stunted, did not mature and developed black pustules typical for VK. Leaf symptoms were not visible under greenhouse conditions.

Restriction profiles resembling the MLO-type II (Stolbur-like) pattern according to Ahrens and Seemüller (1992) could be achieved with DNA fragments amplified from *C. arvensis*, *S. nigrum* and *H. obsoletus*, all collected in vineyards, and from grapevine seedlings on which the planthoppers were fed (Figure). Samples from VK affected vines collected in the field led to identical patterns (Maixner *et al.* 1994). No MLO-specific restriction profiles could be achieved from laboratory reared healthy *Fiberiella florii* as well as healthy grapevine seedlings. DNA was not amplified with samples prepared from seedlings of *C. arvensis* (data not shown).

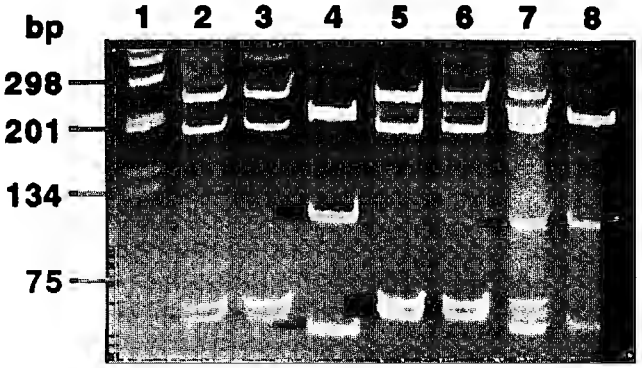

Figure: Polyacrylamide gel electrophoresis analysis of *AluI* digested PCR products (16S rDNA fragments) obtained after 40 cycles. (1) Molecular weight standard; (2) *Hyalesthes obsoletus*, collected from *Convolvolus arvensis*; (3) *H. obsoletus*, used in transmission experiment; (4) Laboratory reared *Fiberiella florii*, healthy. Unspecific (non-MLO) profile; (5) *Solanum nigrum* with virescence symptoms from a vineyard; (6) *C. arvensis* with stunting symptoms from a vineyard; (7) Grapevine seedling inoculated by *H. obsoletus*. The pattern is a combination of the MLO-specific profile and the unspecific profile visible in (8). (8) Grapevine seedling, healthy. Unspecific (non-MLO) profile.

The detection of MLOs of the same group in weeds, grapevine, and insects is not sufficient to identify *H. obsoletus* as vector of VK, since MLO detection in insects is not necessarily correlated with their ability to transmit the pathogens to plant hosts (Vega *et al.* 1993). Nevertheless, feeding of *H. obsoletus* resulted in typical yellows symptoms in grapevine seedlings and the MLO was detectable in these plants. Infective planthoppers were the only possible source of inoculation.

The results presented here are the first evidence of a vector of a yellows disease of grapevine beside FD. *H. obsoletus* was found preferably on bindweed, *C. arvensis*, a common weed and potential natural source of the MLO associated with VK. *S. nigrum* is possibly a second herbaceous host of the pathogen in the vineyards. The preference of *H. obsoletus* for herbaceous weeds and its bad survival on grapevine could explain the slow spread of VK in the vineyards.

The identification of a vector of VK is an important step to understand the epidemiology of the disease. However, the vector efficiency of this species and its significance for the spread of VK require further investigation. Other planthopper and leafhopper species will be included in these studies.

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